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CONTENTS

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Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

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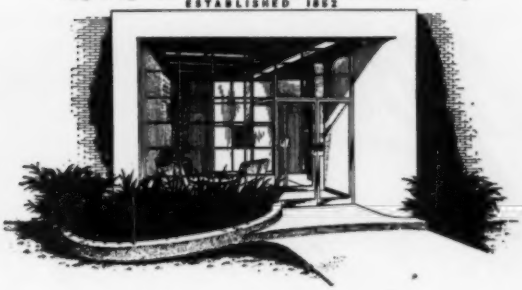

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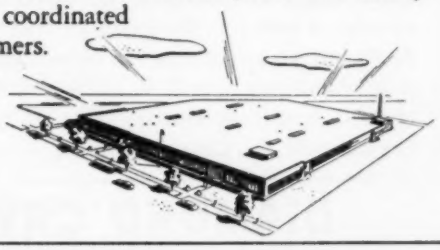

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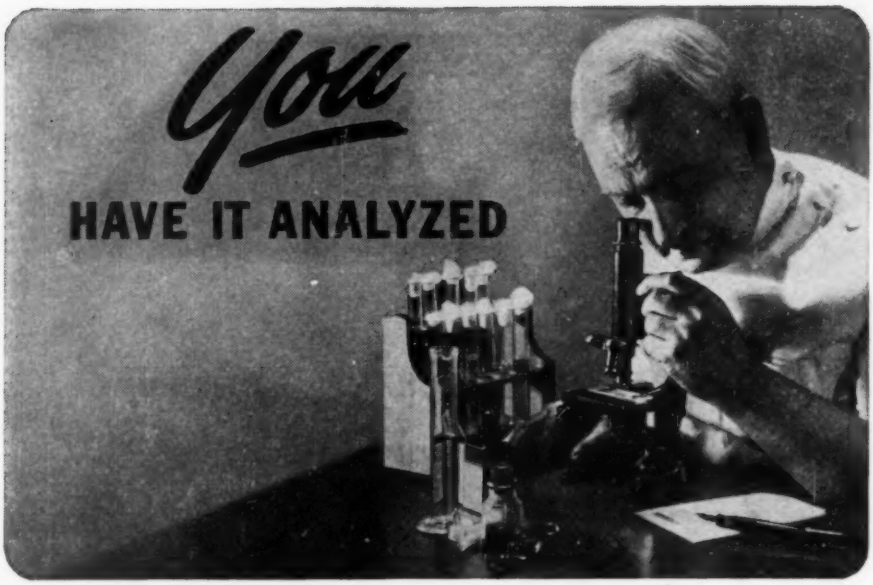

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CEREAL CHEMISTRY

VOL. XXVI NOVEMBER, 1949 No. 6

WHEAT PROTEIN AND THE BIURET REACTION[1]

ALVIN J. PINCKNEY[2]

ABSTRACT

The biuret reaction has been applied to the quantitative estimation of protein in wheat and wheat flour. The procedure is simple, rapid, and inexpensive. The protein, peptized by 0.05 *N* potassium hydroxide solution, is treated with copper sulfate stabilized in alkaline solution by a small amount of glycerol. Color intensity of the reddish-violet product, which is proportional to the protein concentration, is determined with a colorimeter. Approximately one-tenth of the protein remains unpeptized and, therefore, not directly measured. Nevertheless, biuret color values are closely correlated with crude protein values as determined by the Kjeldahl method. It is well known that bread loaf volumes are generally closely correlated with Kjeldahl protein content. In this study, they are shown to be correlated about equally well with biuret color values.

In applying the method, biuret values may be evaluated in terms of either total protein or peptized protein, as desired, by means of a graph, table, or formula derived from the biuret and protein values of suitable test samples.

Protein content of wheat has long been recognized as a useful index of its value for breadmaking purposes. The Kjeldahl method for determining protein is accepted as standard, but certain disadvantages of the method are well known. One of the objectives of the Production and Marketing Administration and of the Bureau of Plant Industry, Soils, and Agricultural Engineering has been the development of a test that is simpler, faster, and less expensive than the Kjeldahl method and at least equally as useful for evaluating wheat. In the search for such a substitute, attention was directed to the biuret reaction, which has long been used as a qualitative test and more recently as a quantitative test for protein in biological materials.

When copper in strongly alkaline solution reacts with protein material, a reddish-violet substance is formed. Under proper condi-

[1] Manuscript received May 17, 1949. Presented at the Annual Meeting, May, 1949.
This study was conducted jointly by the Bureau of Plant Industry, Soils, and Agricultural Engineering and the Grain Branch, Production and Marketing Administration, both of the United States Department of Agriculture.

[2] Chemist, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Beltsville, Maryland.

tions, the intensity of the color produced is proportional to the protein concentration. Copper may react in a similar manner with non-protein materials which contain the amino and carboxyl groups characteristic of proteins.

Wiedemann (1) has been credited with first observing the reaction with biuret, $NH_2CO.\ NH.\ CONH_2$. Ritthausen and Pott (8) discovered in 1873 that proteins also react in this way and shortly thereafter the reaction came into common use and was called the "biuret" test.

The chemistry of the reaction was studied thoroughly in 1896 by Schiff (10). Riegler (7) in 1914 and Autenreith and Mink (2) in 1915 adapted the test to the quantitative estimation of protein in biological fluids.

Riegler used egg albumen in preparing a "comparison liquid" while Autenreith and Mink used purified biuret as a standard. Little use was made of the new method for several years. In 1928 Hiller (5) made some minor changes in the method and confirmed its usefulness. In 1934 Fine (4) made some further refinements, but his chief contribution was the use of blood serum as a standard.

Robinson and Hogden (9) in 1940 studied and summarized the method and have supplied a good review of the literature.

Materials and Methods

In the biuret test as it has usually been employed, copper sulfate and protein are brought together in a solution containing 3% or more of sodium hydroxide. In the presence of alkali in this concentration, most of the copper is quickly precipitated as the hydroxide, but a small amount is maintained in solution as a complex ion. This is essential to the completion of the reaction which is relatively slow, probably because it involves the complex protein molecule.

The peptization of wheat protein is apparently more nearly complete in 0.05 *N* alkali than in alkali of any other concentration. Accordingly, this concentration was used in our first attempts to apply the biuret reaction to wheat protein. A fair degree of correlation with Kjeldahl protein values was noted, but results obtained were so variable as to indicate the probability that in successive tests the reaction was stopped in varying stages of completion by premature removal of copper by precipitation. This difficulty was overcome by the application of Mehl's (6) proposal in 1945 that the precipitation of copper be avoided by the introduction of ethylene glycol. Glycerol, proposed for this purpose by Sols (11) in 1947, proved even more effective as much smaller quantities of it are required.

The following equipment, reagents, and procedure were used throughout this study:

Equipment

1. Balance, analytical.

Samples were weighed with about the same degree of precision as is ordinarily used in the Kjeldahl procedure.

2. Shaker—motor-driven rack which inverts stoppered bottles about 60 times a minute.
3. Centrifuge—Clay Adams "Senior." Speed 4000 r.p.m.
4. Mill—"Labconco."
5. Colorimeter—Klett-Summerson (12) photoelectric. Fitted for 1 cm. cuvettes, parallel face; No. 54 K-S light filter.

Reagents

1. Stock solutions
   (a) Potassium hydroxide
   Prepare a saturated solution, let settle until clear. Dilute the saturated solution to exactly 10.0 *N*.
   (b) Sodium hydroxide
   Prepare a saturated solution, let settle until clear. Dilute to 6%; 100 ml. contains 6 g. of NaOH.
   (c) Glycerol—2% solution
   100 ml. contains 2 g. of glycerol.
   (d) Copper sulfate
   Select only pure crystals of the pentahydrate. Make 4% solution; 100 ml. contains 4 g. of $CuSO_4.5\ H_2O$.
2. For use
   (a) Potassium hydroxide
   Dilute 50 ml. of 10 *N*. to 10 liters—dilute solution is approximately 0.05 *N*.
   (b) Biuret reagent
   Mix thoroughly 60 ml. of 6% sodium hydroxide solution, 160 ml. of 2% glycerol solution, and 740 ml. of distilled water. While stirring vigorously, pipette in slowly 40 ml. of 4% copper sulfate solution. The resulting solution should be clear, deep blue, and stable for several weeks. It should not be used after any precipitate forms and should be stored in an alkali-resistant glass bottle.

Procedure

Mix 2 g. of flour or freshly and finely ground wheat with about 5 ml. of carbon tetrachloride in a bottle. Pipette into the bottle 50 ml. of 0.05 *N*. potassium hydroxide solution and shake for 10 minutes

in a shaker, or by hand if no shaker is available. Centrifuge the protein dispersion or a portion of it until very nearly clear. With accurate pipettes, measure 5 ml. of the clear centrifugate and 15 ml. of the biuret reagent into a test tube. Mix by inverting 3 or 4 times. Let stand for 1 hour or longer, then determine the intensity of color of the reddish-violet solution with the colorimeter. Convert colorimeter scale readings to protein values by means of a chart, table, or formula. (This will be discussed later.)

Discussion

Results obtained by the Kjeldahl method may differ from those obtained by the biuret method as here proposed. In the Kjeldahl procedure, total nitrogen is determined. From this the protein content is calculated with no distinction between protein and nonprotein nitrogen. The biuret reaction involves the peptide linkage and, therefore, ordinarily may be expected to furnish a fairly accurate measure of true protein. As the reaction is here applied, the entire amount of the protein in the sample tested is not directly determined since the peptizing agent leaves unpeptized a small fairly constant fraction of the wheat or flour protein. This portion appears to include chiefly nonendosperm and, therefore, nongluten proteins.

The colorimeter does not distinguish between color intensity and turbidity. If color intensity is to be accurately measured, the colored solution should be as nearly clear as possible. If the lipids present in the wheat and flour are not removed before the protein is peptized, a slight variable turbidity is produced. This turbidity is reduced by removing a major portion of the lipids with carbon tetrachloride, which is in turn eliminated by the centrifugation.

The biuret reagent as here used contains sufficient copper to react with the protein in samples of wheat or flour in which the protein content is not greater than 18%. If it is necessary to test samples in which the protein content is greater than this value, more copper might be included in the reagent, more of the reagent might be used, or smaller samples might be taken. If any of these changes is made, it becomes necessary to prepare a new chart or table for determining the protein values.

The biuret reaction apparently requires about 20 to 40 hours to reach completion. It proceeds rapidly for the first 5 to 10 minutes, then more slowly until at 30 to 60 minutes it is very nearly complete. The change in color intensity is so slow as to be nearly imperceptible during the next 3 hours. Changes of 2 scale units have been noted over a period of 20 hours after the first hour.

Robinson and Hogden (9), Mehl (6), and others have shown that optical densities of biuret test solutions have a maximum value at approximately 550 mμ. This is found to be true also when glycerol is used in the test. This may be seen in Fig. 1. When copper is not permitted to precipitate, as in the presence of glycerol or ethylene glycol, part of the color in the test solution comes from the copper which has not reacted with protein. This part is *zero* if the quantity of protein present is sufficient to combine with all of the copper, and is greatest if *no* protein is present, as in a "blank" test. The color re-

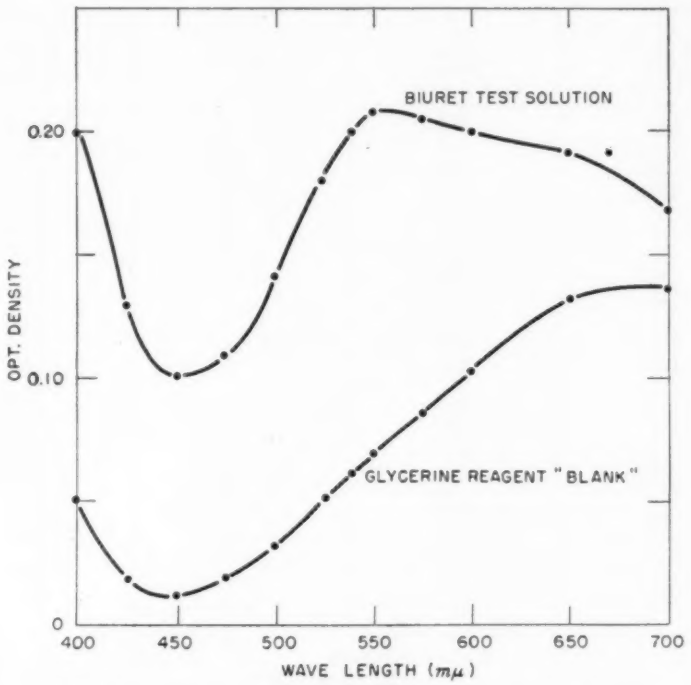

Fig. 1. Optical densities of biuret test solutions at different wave lengths.

sponse of such a test is also shown in Fig. 1. It may be noted that the greatest difference in optical density between "blank" and "biuret" occurs at about 550 mμ. The light filter "K-S No. 54" has a spectral range of approximately 500 to 570 mμ, centered at 540 mμ, and is appropriate for this work. Data for Fig. 1 were obtained by use of a Coleman Junior Spectrophotometer, zero adjustment made with water.

Earlier workers agree that the concentration of sodium hydroxide in the test solution should be no less than 3%. Preliminary experiments in our laboratory indicated that the much lower concentration

of 0.3% is equally satisfactory if ethylene glycol or glycerol is present to stabilize the copper. In Table I are shown the results of an experiment designed to test this observation. Twenty-three samples of wheat were tested as described above. The biuret values of these are shown in column "A." From each of the clear centrifugates prepared for the test a second 5 ml. aliquot was drawn. To it was added 15 ml. of a modified reagent which differed from the usual glycerol-copper

TABLE I

EFFECT OF ALKALI CONCENTRATION IN BIURET TEST SOLUTIONS

"A" NaOH 0.3% K-S scale	"B" NaOH 3.0% K-S scale	Difference	Protein % (Kjeldahl)
132	134	2	15.0
135	135	0	14.2
125	127	2	12.9
137	137	0	15.0
123	125	2	13.1
119	122	3	12.8
121	123	2	13.0
103	109	6	9.9
110	113	3	11.0
99	102	3	10.0
106	109	3	10.4
138	139	1	14.4
115	117	2	12.4
123	125	2	13.2
107	109	2	11.2
106	108	2	11.3
121	122	1	12.6
123	123	0	13.1
140	140	0	15.0
133	132	−1	14.0
130	130	0	13.6
121	122	1	12.5
118	119	1	12.4
	Average difference	1.6	

Correlation, biuret values, and protein		"A"	"B"
	r	0.980	0.972
	$S_{y.x}$	0.30	0.35

reagent only in the content of sodium hydroxide, which was sufficient to make the final concentration 3%. Biuret values thus obtained are shown in column "B." Each set of biuret values has been correlated with the Kjeldahl protein values shown in the third column. It is evident that either reagent will produce satisfactory results.

Material. In connection with another project, several hundred samples, each representing a carload lot of commercial wheat, were taken at the larger grain terminals. From these were selected 100

samples of hard red winter wheat, 36 samples of hard spring wheat, and 28 samples of hard white wheat, representative of the wheat received in 11 markets. The usual range of protein content and the grades of wheat are fairly well covered. Each sample of hard red winter

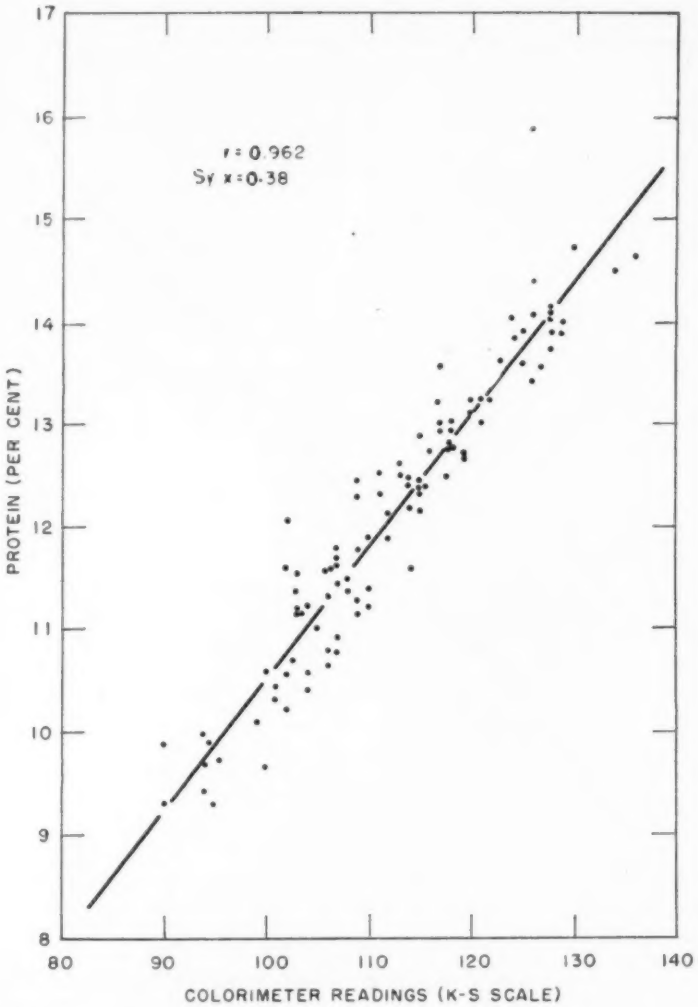

Fig. 2. Correlation of biuret and Kjeldahl protein values of hard red winter wheat.

wheat was milled to 90% patent flour on the Buhler mill. Bread was baked by a formula described by Fifield *et al.* (3) using 100 g. of flour, 2.0 g. of compressed yeast, 1.5 g. of salt, 5.0 g. of sugar, 0.25 g. of malted wheat flour, 3.0 g. of shortening, 4.0 g. of nonfat dry milk solids, and varying amounts (0 to 4 mg.) of potassium bromate for

each loaf. The amount of bromate used was adjusted to produce the maximum loaf value. In most instances the loaf having the greatest volume also had the best grain, texture, and crumb color. The protein content of the wheat and the flour was determined by the Kjeldahl method.

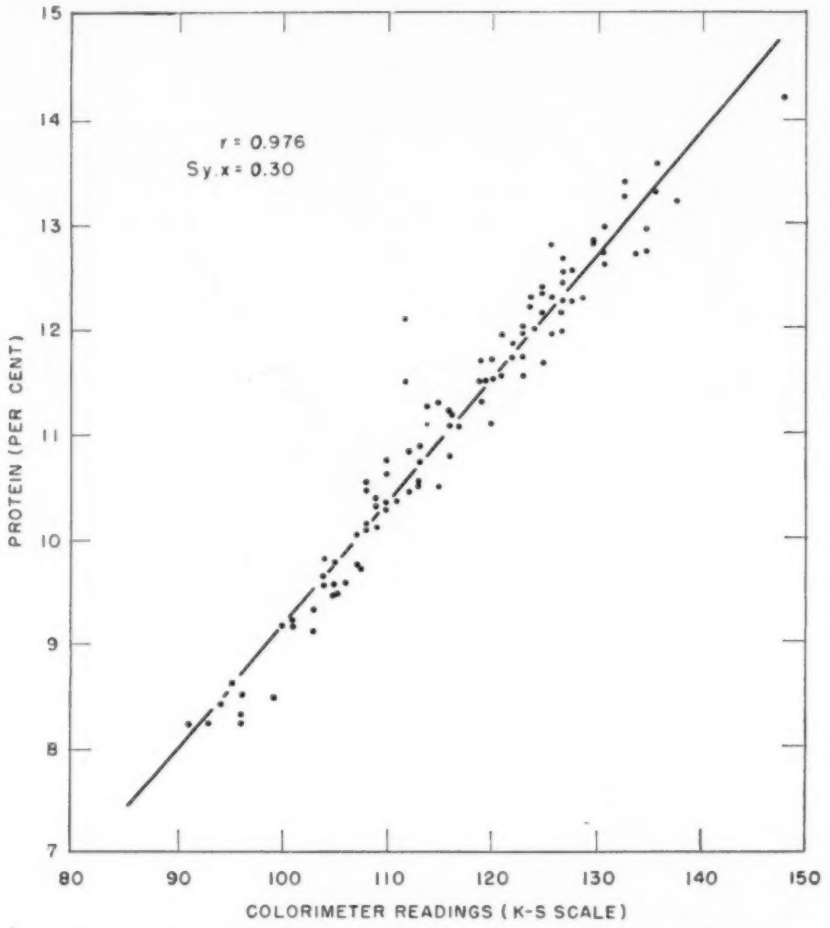

Fig. 3. Correlation of biuret and Kjeldahl protein values of flour milled from hard red winter wheat.

Results

All values presented have been calculated to a 14% moisture basis. Intercorrelations of the different sets of values are shown in scatter diagrams. In Figs. 2, 3, 4, and 5 it may be seen that biuret and Kjeldahl protein values are closely correlated. There is no significant difference, statistically speaking, between the slopes or the positions

of the three lines representing classes of wheat. Obviously the positions of the lines are significantly different from the position of the line representing flour (see Fig. 6).

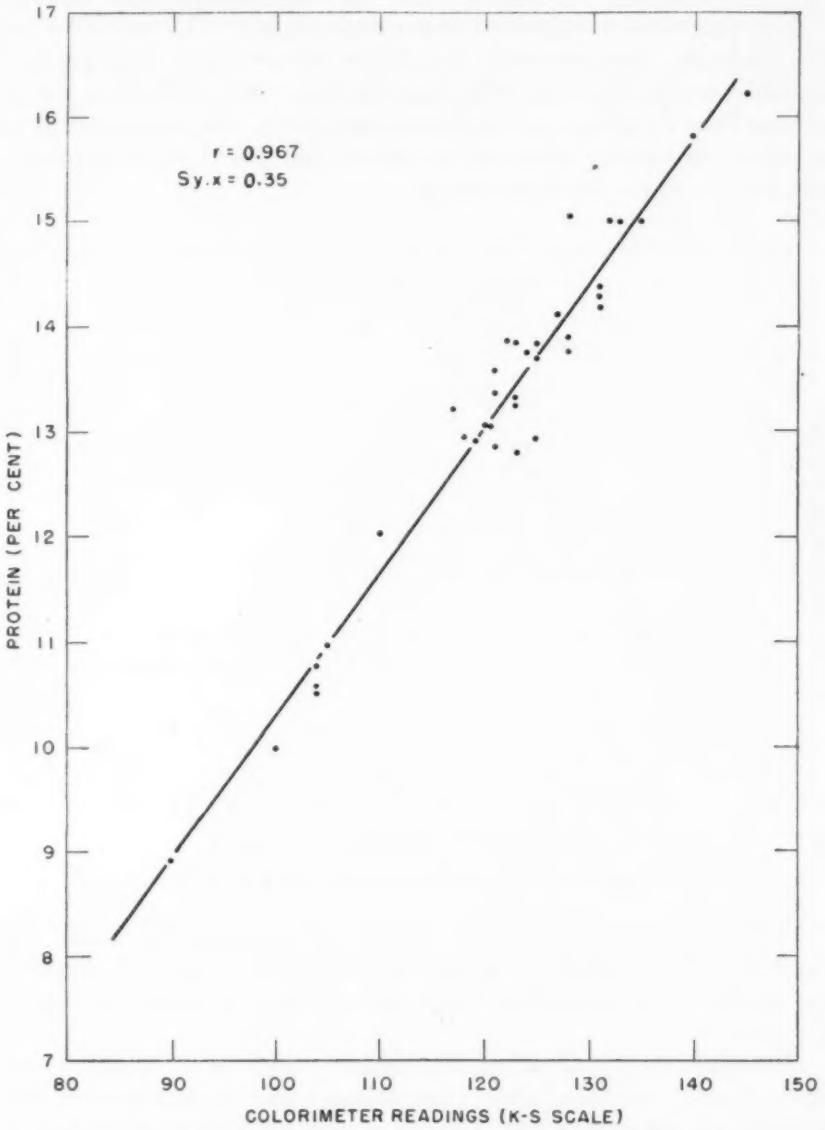

Fig. 4. Correlation of biuret and Kjeldahl protein values of hard red spring wheat.

As previously mentioned, the biuret test as here applied does not directly measure all of the protein contained in wheat or flour. The proportion of protein peptized is greater in flour than in wheat as is

shown by the relatively higher biuret values. In testing 20 of the samples of wheat and corresponding flour, protein was determined in aliquots of the clear centrifugates. Percentages of the total protein represented by the peptized protein were calculated. The averages of these proportions, expressed as percentages, were 85.6 for wheat and 96.8 for flour. Differences in the relative amounts of protein peptized probably account for the differences in slope and position of the regression lines for wheat and for flour, respectively, and may account for the slight differences observed in slopes and positions of regression lines for the three classes of wheat.

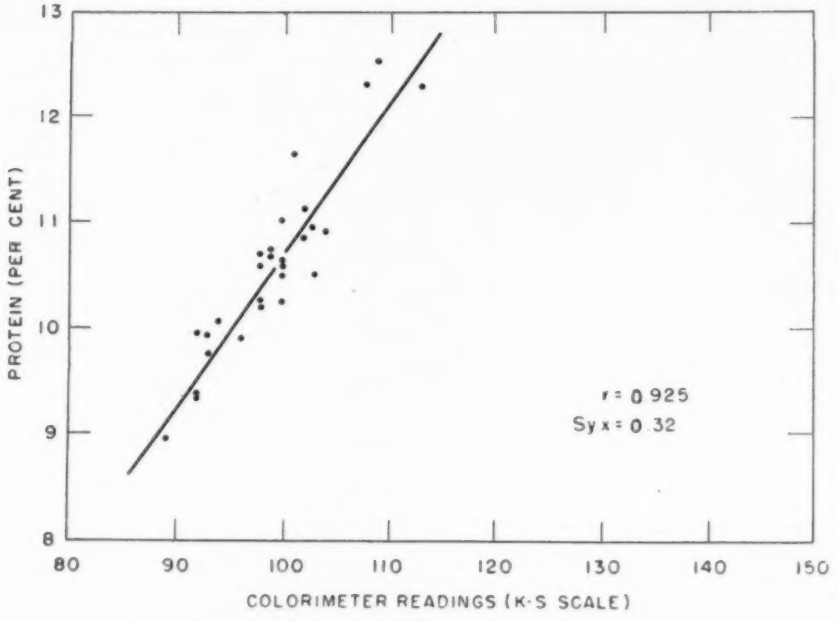

Fig. 5. Correlation of biuret and Kjeldahl protein values of hard white wheat.

Additional evidence that the biuret test compares favorably with the Kjeldahl procedure as a measure of protein content is found in the similarity of correlations with loaf volumes as shown in Figs. 7, 8, 9, and 10.

Reproducibility. Most of the biuret color determinations were made singly. Slightly better correlations would probably have been obtained by the use of the averages of duplicate determinations. In order to demonstrate that good agreement of duplicates can be obtained by this procedure, 22 of the determinations were repeated. As shown in Table II, the average difference between duplicate values obtained is only 0.8 scale unit. Evaluated in terms of protein, this

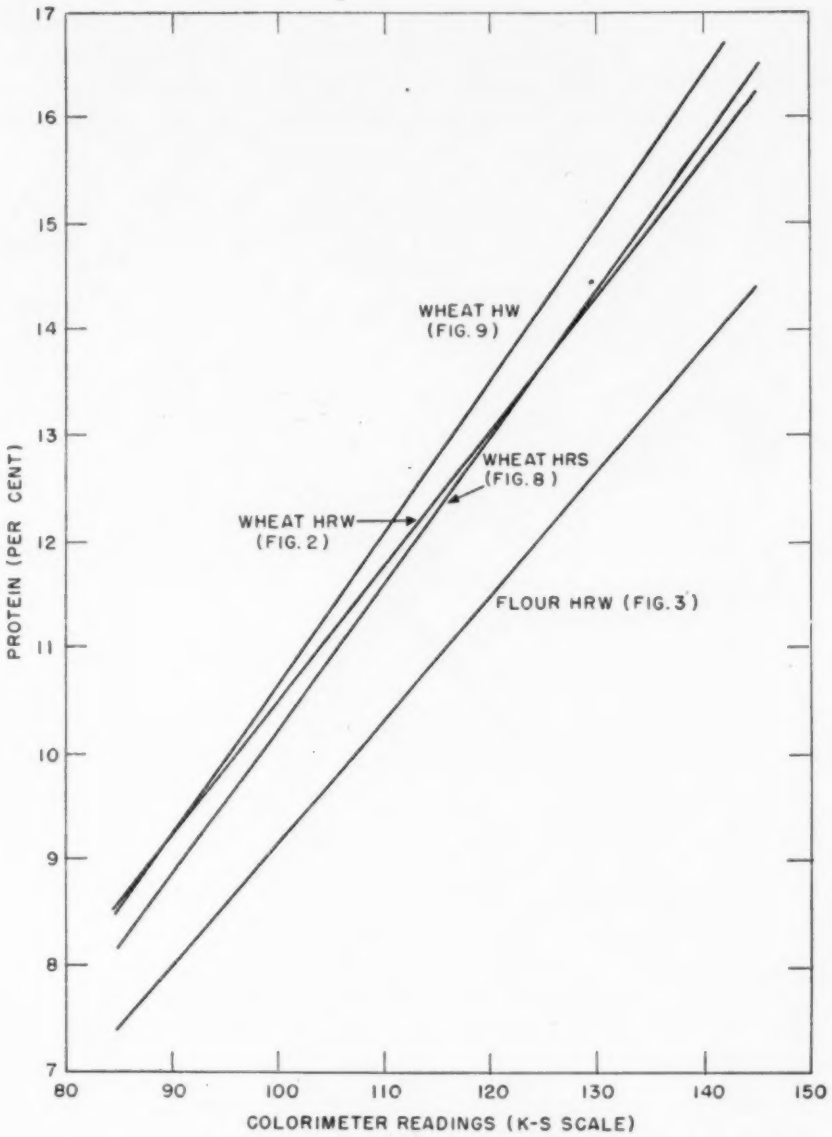

Fig. 6. Regression lines of biuret and Kjeldahl protein values of the three classes of wheat and flour milled from hard red winter wheat.

difference is approximately 0.1%, which compares favorably with the agreement normally expected from the Kjeldahl or other standard procedure.

Application. It is suggested by the data presented in this study

that biuret values may be expressed either (1) as total (Kjeldahl) protein or (2) as protein peptized by dilute alkali. Biuret values expressed in terms already familiar might be more acceptable to cereal workers but could hardly be used interchangeably with values ob-

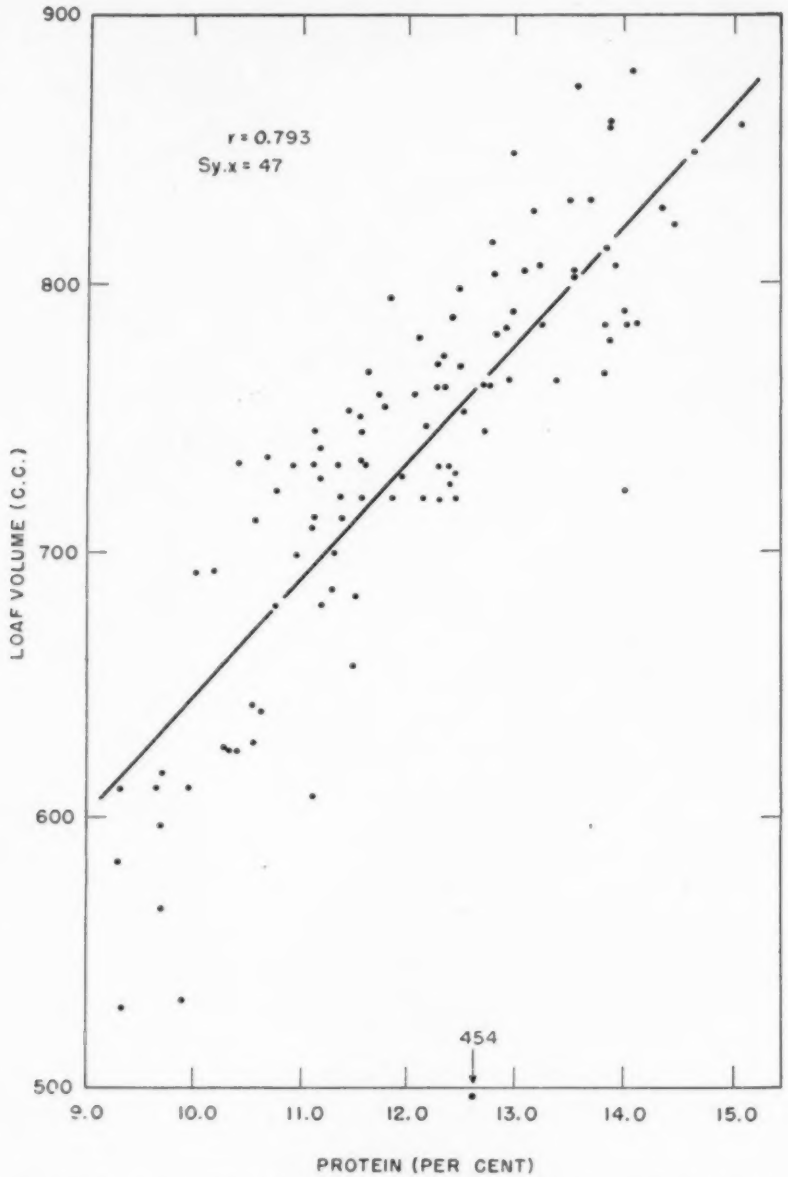

Fig. 7. Correlation of Kjeldahl protein values of hard red winter wheat and loaf volumes.

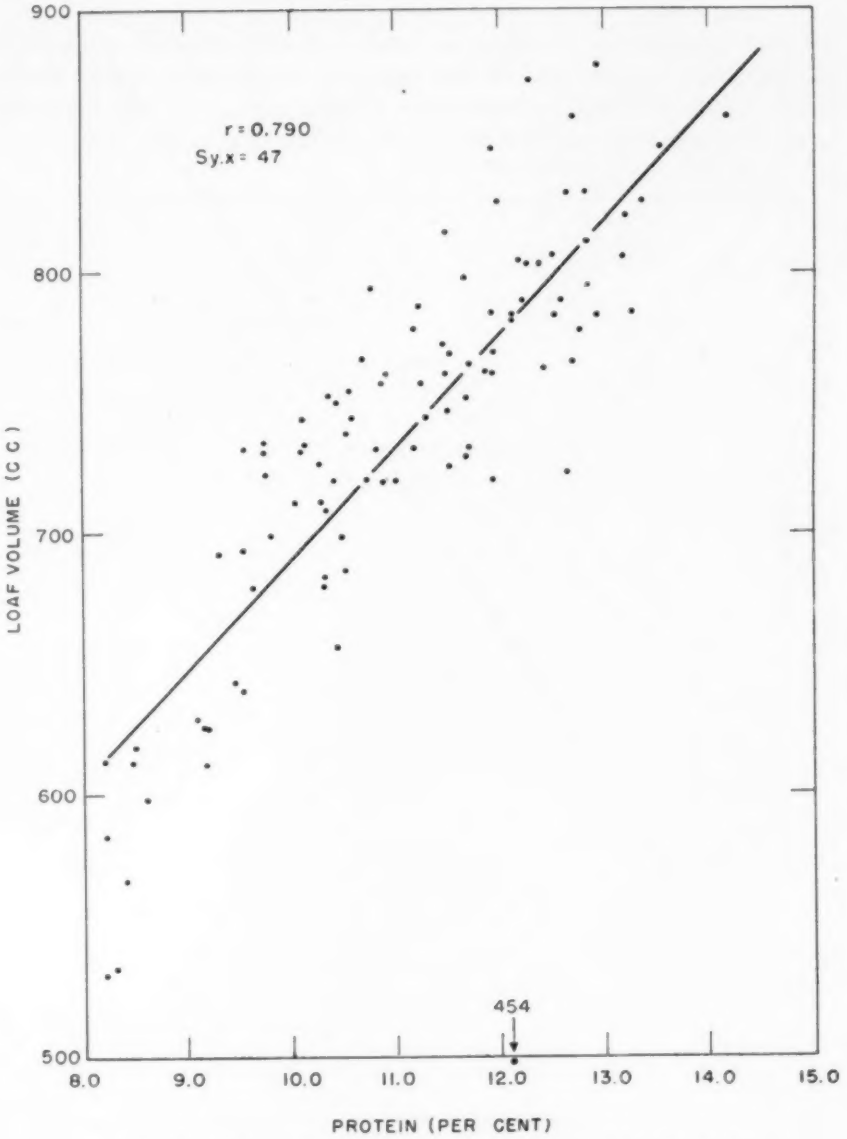

Fig. 8. Correlation of Kjeldahl protein values of flour milled from hard red winter wheat and loaf volumes.

tained by the Kjeldahl method. The values are more accurately expressed as protein peptized by dilute alkali and might be called "Biuret gluten" or some similar name. Conversion of colorimeter scale readings to "Biuret gluten" values would require but one calibration for both wheat and flour.

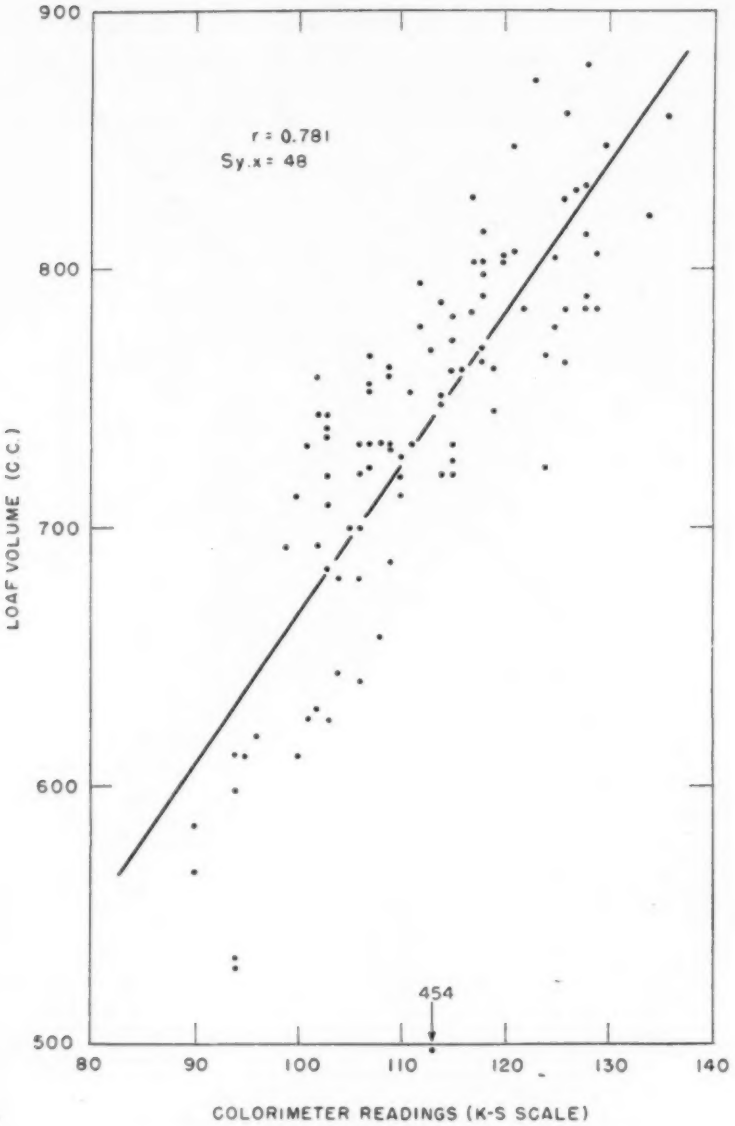

Fig. 9. Correlation of biuret values of hard red winter wheat and loaf volumes.

In general, the scale readings of different colorimeters are not interchangeable, so that each instrument must be separately calibrated. This may be done by determining biuret and protein values of suitable test samples. There may be 30 or more samples of wheat or flour

selected so that the protein values are uniformly distributed over the usual range. Protein values may be determined by the Kjeldahl method, either as total protein or as peptized protein ("Biuret gluten") as desired. From the biuret and protein values a calibration chart

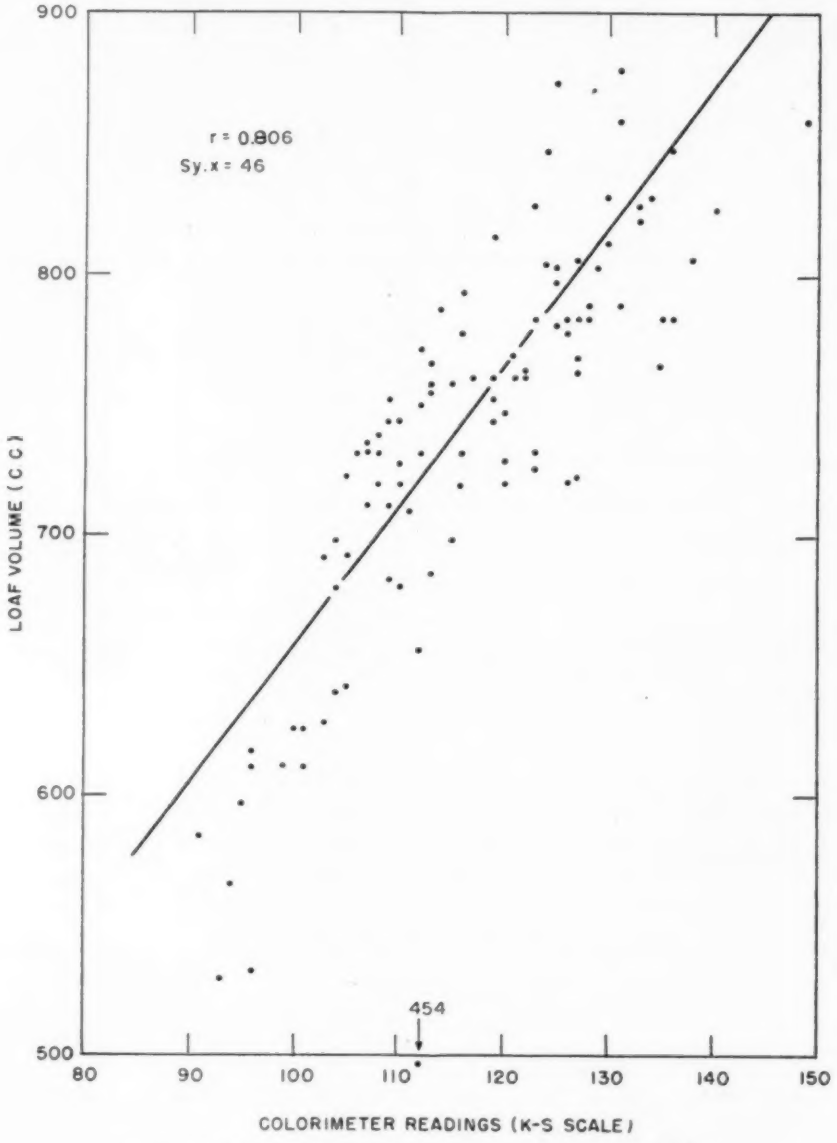

Fig. 10. Correlation of biuret values of flour milled from hard red winter wheat and loaf volumes.

TABLE II

COMPARISON OF DUPLICATE BIURET DETERMINATIONS

KLETT-SUMMERSON SCALE		
"A"	"B"	Difference
128	127	1
126	126	0
148	148	0
119	120	1
129	131	2
120	121	1
105	106	1
105	105	0
125	125	0
100	102	2
112	113	1
131	133	2
106	106	0
107	107	0
136	137	1
126	128	2
135	135	0
134	135	1
128	129	1
97	97	0
105	107	2
102	102	0

Average difference is 0.8 scale unit, equivalent to 0.1% protein.

TABLE III

SUMMARY OF STATISTICS

KJELDAHL PROTEIN CORRELATED WITH BIURET COLOR					
		Flour	Wheat		
		HRW	HRW	HRS	HWh
Corr. coef.	r	0.976	0.962	0.967	0.925
Std. error of estimate	$S_{y.x}$	0.30	0.38	0.35	0.32
	N	100	100	36	28

LOAF VOLUMES CORRELATED WITH:					
		Biuret color		Kjeldahl protein	
		Flour	Wheat	Flour	Wheat
	N 100				
Corr. coef.	r	0.806	0.781	0.790	0.793
Std. error of estimate	$S_{y.x}$	46	48	47	47

(such as Fig. 2), table, or formula may be derived for converting colorimeter values to protein values.

Acknowledgments

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STUDIES ON BREAD STALING IV. EVALUATION OF METHODS FOR THE MEASUREMENT OF CHANGES WHICH OCCUR DURING BREAD STALING [1]

C. W. BICE [2] and W. F. GEDDES [3]

ABSTRACT

A modified Farinograph technique and an empirical crumbliness test have been developed for measuring changes that occur in bread as it stales. The Farinograph method comprises measurement of the decrease which occurs in the consistency of crumb "dough" at constant moisture (62.5%); the crumbliness test involves determining the percentage of crumb which passes through a gyrating sieve (0.25 in. mesh) under controlled conditions.

Crumb "softness" (deformation under constant load), swelling power, soluble starch, Farinograph consistency and crumbliness values on a uniform lot of commercial white bread over a 72-hour period, when interpreted in the usual manner, appear to change at different rates as bread stales. The crumbliness values showed the greatest over-all change followed by crumb "softness," the ratios of the highest to the lowest values being 7.1, and 4.2, respectively, as compared with ratios between 1.3 and 1.9 for the other measures. Crumb "softness," Farinograph and swelling power values decreased in a curvilinear manner with time.

Crumb "softness" curves assume the shape of an equilateral hyperbola and cannot readily be interpreted as indices of staling since their slopes are complicated functions of the rate of change with time and of the original softness of the bread. Many conclusions which have been drawn in the literature from crumb "softness" measurements, the most widely used method for following the changes in bread as it stales, may well be erroneous. On the other hand, crumb "firmness" readings (load required to produce a given compression) increase in nearly linear fashion over the customary three- or four-day period of measurement and their slopes are simple direct functions of the relative rates of change in firmness. Crumb "firmness" can be measured with the Baker compressimeter with more precision than crumb "softness."

Fresh, white bread crumb did not obey Hooke's law when compressed and exhibited appreciable plastic flow; as the bread staled the relation between load and deformation became more nearly linear up to higher and higher loads. As a consequence, the choice of the fixed load or fixed compression employed in crumb "softness" or crumb "firmness" measurements will influence the apparent rate of change in these properties.

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This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 268 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

The data in this paper are to be included in a thesis to be submitted to the University of Minnesota by C. W. Bice in partial fulfillment of the requirements for the Ph.D. degree.

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Staleness, as it applies to bread, is a generic term covering a number of ill-defined changes that occur in bread as it ages. Consumers judge the staleness of bread by direct perception and their subjective estimates probably represent an unconscious integration of many properties. Any physical or chemical measurement would appear to be, at best, only one factor in the subjective estimate, and the relative merits of various objective measurements can only be properly evaluated by a suitably trained and calibrated test panel.

The extensive laboratory and technological researches which have been carried out on the problem of bread staling have been reviewed recently by Geddes and Bice (14) as an aid to investigations supported by the Quartermaster Food and Container Institute for the Armed Forces. Since the staling of bread represents a complex transformation which is not clearly understood, it has been customary to evaluate the degree of staleness by measuring one or more of the several progressive changes which occur in ordinary bread during aging. The crumb changes which have been measured most frequently are the familiar decreases in softness, swelling power and soluble starch. Since the most obvious change in bread during staling is the development of a relatively rigid crumb structure and as the softness of bread appears to be the criterion used by the consumer as an index of freshness, measurement of the compressibility of the crumb has been the procedure most commonly employed for following the staling process.

While relatively few workers have used more than one or two of the available methods in a particular study, there are indications in the literature that the various methods do not give the same relative results (12, 16, 18).

Preliminary to an investigation of the biochemical changes which occur in bread during aging, a critical study was made of the most promising methods for evaluating gross changes in the physical properties of bread. During the course of these researches, a modified Farinograph method and an empirical crumbliness test were developed and compared with the results of compressibility, swelling power and soluble starch procedures on the same lot of bread. A careful examination was also made of the relative merits of the measurement of crumb "softness" (deformation under constant load) and crumb "firmness" (load required to produce constant deformation). These investigations are reported in the present paper.

Modified Farinograph Technique for Following the Staling of Bread Crumb

Fuller (13) investigated the use of the Farinograph as a convenient measure of the swelling power of the crumb, one of the properties

which has long been used as an index of the changes which occur during staling. In his first experiments, he found that when known weights of bread crumb and water were mixed in the Farinograph, the arbitrary consistency figure was lower for stale than for fresh bread. A subsequent modification consisted of titration of a given quantity of bread crumb with water in the Farinograph to give a standard consistency of 500 Brabender units and recording of the total moisture content. As the bread staled, less water was required to yield a "dough" of this standard consistency, and the decrease in absorption was taken as a measure of the staling rate.

Studies with this method made here showed that the results were markedly influenced by the rate at which the water was added, and the replicability of the test was poor. It was noted that very fresh bread crumbs, even without the addition of water, would mix to form

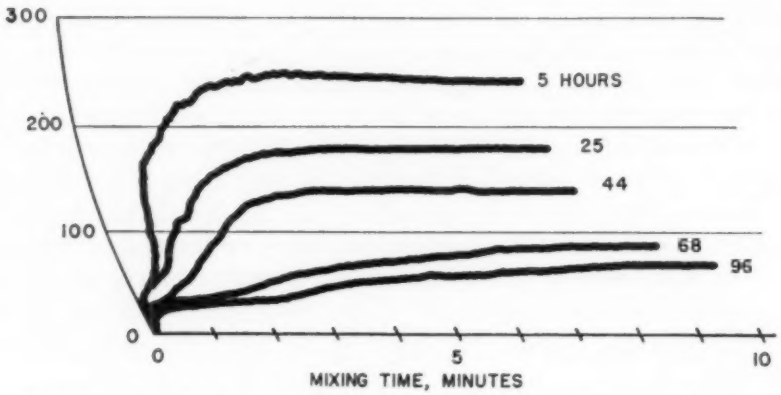

Fig. 1. Curves showing the progressive decrease in Farinograph maximum consistency values of ordinary bread crumb during storage.

a dough in the Farinograph. The recorded curve was smooth and gave a definite, reproducible maximum. Upon addition of water and mixing, stale crumb also formed a dough and gave curves showing a lower maximum consistency than the crumb doughs made from fresh bread.

The minimum amount of water necessary to form a dough with very stale crumb was determined and used as a standard total moisture content for both fresh and stale bread crumb. The modified Farinograph method finally developed was as follows:

A 30 g. sample of crumb (dry weight basis) is made up to 62.5% moisture content with distilled water in the 80 gram Farinograph bowl, and mixing at 30°C. begun immediately. Within 5 to 10 minutes a maximum consistency is reached and is recorded in Brabender units.

Figure 1 illustrates the typical progressive decrease of maximum Farinograph consistency values of bread crumb during staling at room temperature.

Table I illustrates the change in maximum Farinograph readings when the above method was used to follow changes during storage in commercial white bread, laboratory-prepared white bread and canned white bread. The data for all samples indicate a progressive decrease in crumb-dough consistency over the period studied.

TABLE I

CHANGES IN FARINOGRAPH CRUMB-DOUGH CONSISTENCY OCCURRING IN SEVERAL WHITE BREADS DURING STORAGE IN AIR-TIGHT CONTAINERS AT LABORATORY TEMPERATURE

Commercial		Laboratory-prepared		Canned	
Age of bread	Farinograph reading	Age of bread	Farinograph reading	Age of bread	Farinograph reading
hrs.	*B.U.*	*hrs.*	*B.U.*	*hrs.*	*B.U.*
3.3	340	5.0	240	3.0	260
7.0	350	5.2	240	26.0	160
8.0	330	25.0	180	49.0	180
11.0	300	44.0	140	49.5	130
18.2	270	44.2	140	97.5	100
19.3	270	68.0	90	98.0	100
24.0	250	96.0	80	147.0	60
48.0	210			267.0	60
72.0	180			361.0	50
96.2	150				

The possible effect of pH variations on the maximum Farinograph consistency of crumb doughs at constant moisture was investigated by adjusting the pH of the doughs to various levels with solutions of hydrochloric acid and sodium hydroxide. The maximum consistency readings were virtually uninfluenced over a pH range of 1.6 to 7.4. Since the pH of bread normally lies between 5.0 and 5.6, these limited experiments indicated that variation in pH would not be an interfering factor in the application of this technique to measurements made on ordinary bread.

Development of a Method for Measuring Crumbliness

It has long been known that the tendency of bread to crumble when sliced increases upon staling and Selman (24) has recently developed an arbitrary method of measuring the crumbliness of cakes. The method involves the vertical oscillation of cake crumb in a cylindrical device fitted with a sieve, and determination of the quantity of crumb which falls through the sieve. Preliminary studies in this laboratory indicated the possibility of utilizing the gyrator sifter of an

Allis-Chalmers experimental mill in developing an empirical crumbliness test. The effect of crumb surface, sieve mesh opening and of the speed and time of sifting were investigated and the empirical crumbliness test described below was evolved.

A square box with tight fitting slide-on cover and removable sieve supported within, was designed to fit into the gyratory bolter of an Allis-Chalmers experimental mill. The inside dimensions of the box

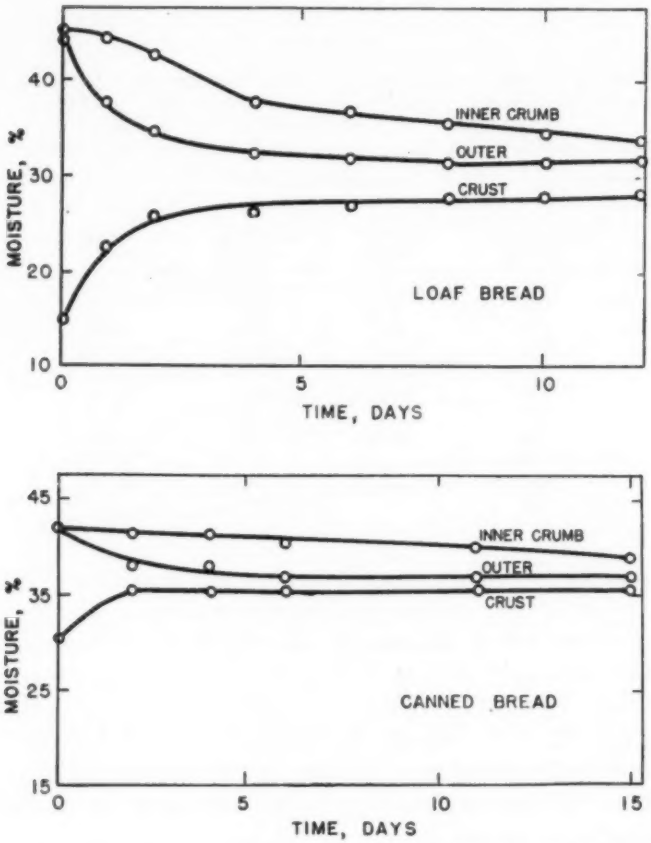

Fig. 2. Curves showing the change in moisture content of crust, inner crumb and outer 0.5 inch of crumb of ordinary white loaf bread and canned white bread during storage at room temperature.

were $3 \times 13 \times 13$ in. The lid was held in place by metal flanges along two sides of the box. The effective sieve area was one square foot, and the mesh, ¼ by ¼ in. Bread crumb slices 1¼ in. square and 0.5 in. thick were used for the determination of crumbliness. A 50 g. sample ("as is" basis) of the crumb slices was placed in the covered sifter box and shaken at 270 r.p.m. for 10 minutes in a room maintained at approximately 25°C. and 75% relative humidity. The

crumb passing the sieve was collected, dried in a forced draft oven at 130°C. for one hour and weighed. Data are reported as per cent of the original sample (dry basis).

Distribution of Moisture in Bread Crumb

When bread is stored, it seems quite likely that the crumb loses moisture to the relatively dry outer crust; such a decrease in crumb moisture would set up gradients which might be of sufficient magnitude to influence the compressibility and crumbliness. That such a moisture gradient does exist was shown by determinations of the moisture content of the crust, outer 0.5 in. of crumb, and inner crumb of ordinary white bread (experimental loaves baked from 150 gram doughs) and of canned bread (No. 2½ tins) made by the regular army formula. Measurements were made after allowing the loaves to stand for 12 to 15 days in air-tight containers at room temperature. The results presented graphically in Fig. 2 show that immediately after baking, the inner and outer crumb have the same moisture content whereas the crust is considerably drier; upon storage, the outer crumb loses moisture to the crust thereby setting up a moisture gradient in the crumb which still existed after a period of 12 to 15 days.

Comparative Rates of Changes in Various Properties of Bread

After experience had been gained with various procedures, a comparative study was made of the compressibility, swelling power, "soluble starch" (water-extractable polysaccharides), Farinograph and crumbliness methods for measuring the gross changes which occur in bread as it ages.

For this study, 60 1.5 lb. loaves of white bread were obtained direct from a commercial bakery and allowed to cool to room temperature. To lessen the possibility of the establishment of a moisture gradient, during storage, the crust and outer 0.5 inch of crumb were removed from half of each loaf and the remaining crumb was then shredded by a 10-second treatment in the Waring Blendor, combined and sealed in cans to provide a representative uniform sample for Farinograph, swelling power and soluble starch determinations. The remaining halves of each loaf were sliced and the crust and outer 0.5 in. of crumb removed as before. Alternate slices were then separated and stored, at room temperature in a definite order for compressibility and crumbliness determinations, respectively. The slices for the crumbliness study were further cut into 1¼ inch squares before being sealed in airtight containers. Measurements by the various methods were made 3, 7, 11, 18, 24, 48, and 72 hours after baking.

Crumb compressibility values were obtained by means of a Baker compressimeter using a pressure plate 1.0 in. in diameter and crumb slices 0.5 in. thick and three in. square. The compression in mm. under a 20 g. stress was recorded.

Swelling power was determined by the modified method of Schoch and French (1, 22).

"Soluble starch" was precipitated by means of absolute ethanol from the supernatant crumb extract obtained during the swelling power determination. The procedure was similar to that applied by Schoch and French (22). Data were recorded as per cent dry matter obtained from the original crumb (dry basis).

Maximum Farinograph consistencies and crumbliness were determined according to the modified techniques already described in this paper.

TABLE II

STALENESS CHANGES IN COMMERCIAL WHITE BREAD AS MEASURED BY SEVERAL METHODS

Age of bread	Crumbliness	Compressibility	Farinograph consistency	Swelling power	Soluble starch
hrs.	*%*	*mm.*	*B.U.*	*grams*	*%*
3	7.6	2.1	340	4.25	4.5
7	10.5	2.1	340	4.14	—
11	13.1	2.0	300	3.93	4.0
18	20.2	1.1	275	3.72	3.8
24	23.4	0.8	250	3.53	4.0
48	39.4	0.6	205	3.24	3.6
72	54.1	0.5	175	3.02	3.4
Ratio of highest to lowest value	7.1	4.2	1.9	1.4	1.3

The results summarized in Table II represent the means of duplicate determinations, with the exception of the compressibility figures which were the means of readings on 15 slices of bread.

This experiment was conducted several years ago and the data are presented to demonstrate not only the comparative results but also some of the apparent difficulties and disadvantages underlying their use and interpretation.

Interpreting these data in the usual manner would lead to the conclusion that the crumbliness values showed the greatest over-all change and that crumb "softness," Farinograph, swelling power and soluble starch values showed progressively less over-all changes. This would suggest that the different methods do not indicate the same extent of staleness over the 72-hour period. Further, when the changes for each time interval expressed as a percentage of the total change for each respective method were plotted against time (Fig. 3)

the various methods also appeared to show different rates of staling. Thus, 80% of the total change in compressibility value over the 72-hour period occurred within the first 24 hours and thereafter the change was very gradual. Crumbliness on the other hand, showed very nearly a linear change with time.

The Farinograph consistency and swelling power methods gave results which changed in a curvilinear manner with time. The curve for the soluble starch data is not shown on the graph, but it was found to lie slightly above the swelling power curve and to be curvilinear.

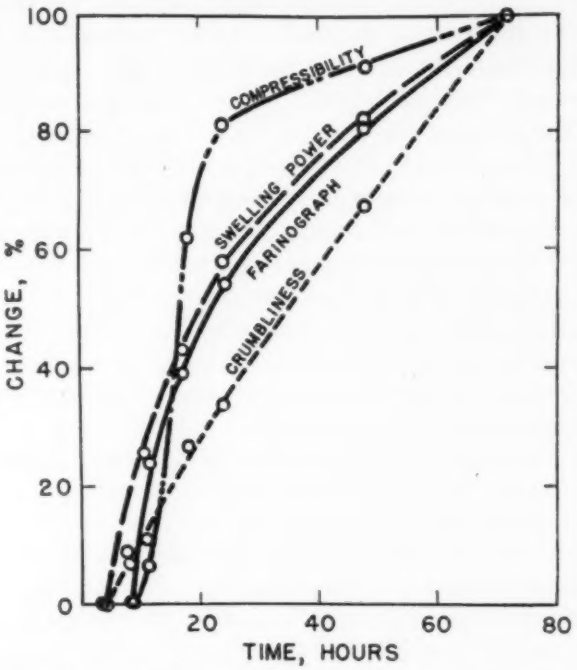

Fig. 3. Curves showing the per cent change in several properties of bread crumb during staling.

As will be shown later, the comparison of staling data is not as simple as would be indicated by the usual treatment above.

Measurement of Crumb Compressibility

The development of a relatively rigid crumb structure as bread ages provides a mechanical basis for following and recording the staling process in numerical terms. Several devices for measuring the compressibility of bread crumb have been employed by different workers (2, 3, 4–8, 9, 16, 18–21, 23, 25). Of these, the Baker compressimeter (1, 21) was especially designed for measuring the compression which occurs when a flat disc is pressed against the surface

of the crumb under a given load; since the force is progressively applied by means of a lever and windlass operated by a synchronous motor, a series of force and deformation values can be read from the scales which are provided.

Crumb hardness changes in bread crumb have been measured in two ways—either by noting the force required to give a standard compression value; or by observing the compression obtained upon application of a standard force. In the first instance, greater and greater loads are required with time to produce a standard deformation, and it is logical and convenient to call the values "firmness" readings. However, in the second method, smaller and smaller deformation readings are obtained with time for a given standard force and they may be termed "softness" data. The vast majority of workers have measured the compression produced by a constant load but recently Favor and Johnston (11) and Thomas (26) have measured the force required to produce a constant deformation in studies of the effects of certain ingredients and other variables on the rate of staling of bread. They found that certain ingredients delayed the rate at which the bread became firm, a conclusion which is at variance with that of Sumner and Thompson (25) who determined the compression produced under a constant load. The relative merits of crumb "firmness" and "softness" measurements thus appear to require careful examination.

Crumb compressibility values are naturally influenced by such factors as the moisture content of the crumb and its distribution and by the size and thickness of the cell walls. The crumb grain, and also the moisture content, may vary in different sections of the loaf and it is generally realized that the replicability of the test is rather poor although quantitative data are limited to the studies of Freilich (12) with bread and of Noznick and Geddes (17) with cake.

Platt (19) subjected bread crumb, three hours out of the oven, to several different loads and found that the deformation varied directly with the magnitude of the load; that is, it followed Hooke's law and behaved as a perfectly elastic body. The question whether bread crumb follows Hooke's law is of such theoretical and practical interest that further investigations of the relation between stress and strain should be made. If this law holds for bread, the crumb "firmness" can readily be computed from crumb "softness" data and vice versa; moreover, these results could be expressed in terms of a modulus of elasticity, a constant which is characteristic of the material and entirely independent of the stresses (or strains) employed in carrying out the measurements.

It must be emphasized that cereal chemists and physicists use the term "compressibility" in an entirely different sense. In physics, compressibility is the reciprocal of the bulk modulus, the modulus of elasticity which applies only in cases where the force or pressure is brought to bear equally on all sides, or over the whole surface of the test object. The bulk modulus is not applicable to bread compressimeter measurements where the pressure or force is applied only to two faces of the bread section. The most appropriate modulus of elasticity in such cases, provided Hooke's law is followed and shearing does not occur, is Young's modulus (the modulus of stretch) in which by definition, equal force is exerted at opposite ends of the test object. Young's modulus = force per unit area/change in length per unit length; that is,

$$Y = \frac{F/A}{e/L} = \frac{FL}{Ae},$$

where, in the case of bread, F is the force applied; A, the effective area of the pressure plate; e, the decrease in slice thickness; and L the initial slice thickness. Young's modulus, stress per unit strain, has been used in the cereal chemical literature (17, 19) as a modulus of compressibility. Since crumb "firmness" readings are force values they are directly proportional to Young's modulus or to the modulus of compressibility as defined in the cereal chemical literature.

Theoretical and experimental observations concerning the various aspects of compressibility methods for following bread staling are presented in the sections which follow.

Crumb "Firmness" vs. Crumb "Softness" as Measures of Staling Rate

An indication of the most appropriate method of expressing and interpreting crumb compressibility data may be obtained from a theoretical consideration of the behavior of bread under compression.[4] For the sake of simplicity, it will be assumed that bread satisfies Hooke's law when compressed.

Let F = the actual force applied to the pressure plate of the compressimeter, and let σ = surface area of pressure plate, d_0 = original thickness of crumb, d = actual thickness of crumb under load. Then stress, $Z = F/\sigma$; and strain, $\epsilon = (d_0 - d)/d_0 = \delta/d_0$.

If Hooke's law is satisfied

$$Z = E(t).\ \epsilon. \qquad (1)$$

The modulus, $E(t)$, is similar to Young's modulus and in order to take care of changes due to staling it is assumed to be a function of

[4] The authors are indebted to Professor Andrew Hustrulid, Physicist, Division of Agricultural Engineering, University Farm, for the theoretical treatment presented here.

time. If the isometric [5] (ϵ = constant) is a straight line, then $E(t)$ is a linear function of t and may be written

$$E = E_1 t + E_2. \tag{2}$$

E_2 is a measure of the initial condition of the crumb. If E_1 is defined as a measure of the rate of staling, it can be found easily by experimentation. It is simply $1/\epsilon$ times the slope of the isometric.

$$\frac{1}{\epsilon}\left(\frac{\partial Z}{\partial t}\right)_\epsilon = \frac{1}{\epsilon}\frac{\partial}{\partial t}\left[(E_1 t + E_2)\epsilon\right] = E_1. \tag{3}$$

In practice, crumb "firmness" curves represent a plot of the force applied to the compression plate to produce a constant compression (δ) as a function of time. Equation (1) may be written:

$$F = \frac{\sigma}{d_0} E(t)\ \delta, \text{ when } F = \text{"firmness" or load.} \tag{4}$$

The slope of the "firmness" curve is therefore proportional to the rate of staling as defined above, since

$$\left(\frac{\partial F}{\partial t}\right)_\delta = \frac{\sigma\delta}{d_0} E_1 = K\ E_1.$$

The rates of staling of different breads can be evaluated easily by comparing the slopes of the "firmness" curves which are obtained for constant values of σ, d_0 and δ.

When δ is measured as a function of t for a constant load, F, it is a measure of crumb "softness" and the question arises whether the rates of staling of different breads can be obtained by comparing the slopes of the "softness" curves.

From equation (4)

$$\delta = \frac{d_0 F}{\sigma E(t)} = \frac{d_0 F}{\sigma(E_1 t + E_2)} \tag{5}$$

and

$$\left(\frac{\partial \delta}{\partial t}\right)_F = \frac{-\ d_0 F}{\sigma}\frac{E_1}{(E_1 t + E_2)^2}. \tag{6}$$

It is obvious from equation (6) that a measure of E_1 for different breads cannot be obtained by comparing the slopes unless they had the same modulus initially; that is, unless E_2 is the same for all breads being compared. Even then the task would be difficult and the results uncertain because the slopes are constantly changing with time. Within the limits of the assumptions that the force, F, is proportional

[5] The isometric is the "curve" representing the value of Z as a function of time for conditions of constant strain.

to the compression, δ, and that the rate of staling, E_1, is constant, it must be concluded that the relative slopes of "firmness" curves provide the only satisfactory measure of comparing staling rates from compressibility data.

The question whether one should use "firmness" or "softness" units is therefore not purely an academic one, especially when attempts are made to reach conclusions regarding relative staling rates of two or more breads by a mere visual examination of the curves, the practice which has been followed by most workers in this field. "Softness" curves drawn through a sufficient number of points ordinarily tend to approximate an equilateral hyperbola, whereas, as will be seen later

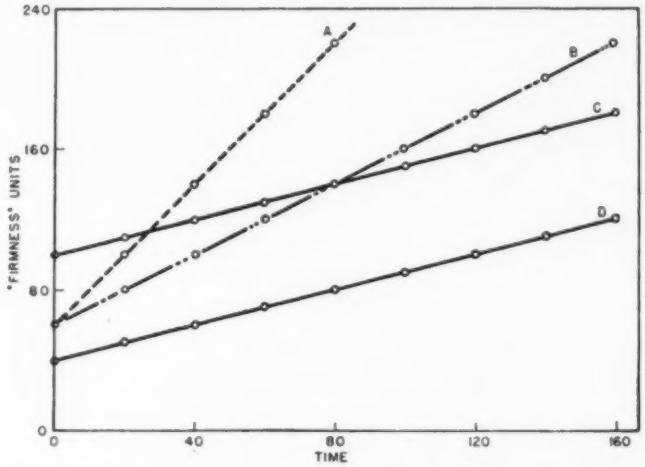

Fig. 4. Ideal "firmness" data plotted against time. General equation for the curves is: $y = 2\,mt + b$, where y = firmness units, t = time, b = y intercept or initial firmness reading, 2 = y scale adjustment since there are twice as many firmness units per linear dimension of ordinate as there are time units per abscissa, and m = the slope, or rate of firmness change. Slopes are as follows: $m_A = 1$, $m_B = 0.5$, $m_C = 0.25$, and $m_D = 0.25$.

in this paper, "firmness" curves are more nearly linear over the three- or four-day period during which staling is normally followed; that is, "softness" is approximately an inverse function of time whereas "firmness" is approximately a direct function of time. "Softness" is approximately the reciprocal of "firmness," the relationship between them being constant only if the original stress-strain data conform to Hooke's law.

The implications of the preceding theoretical treatment of the relative utility of the two methods of expressing the results of compressibility measurements may be demonstrated graphically by plotting hypothetical staling data in which it is assumed that crumb "firmness" increases in a perfectly linear fashion with time. Four

"firmness" curves, the equations for which are known and so chosen as to demonstrate the various characteristics which one hopes to compare by inspection in practical studies, are plotted in Fig. 4. Curves C and D differ in height but have the same slope; thus, "bread" D is "softer" than "bread" C but the rates of change are the same. Curves A and B, on the other hand, have the same original "softness" but A changes more rapidly than B. The reciprocal values of these "firmness" data are plotted in Fig. 5; the four "softness" curves (A′, B′, C′, D′), correspond to the "firmness" curves (A, B, C, D, Fig. 4). These reciprocal values give curves which are displaced equilateral hyperbolae, the shapes of which are characteristic of "softness" curves, appearing in the literature. It is readily seen that the rate of staling

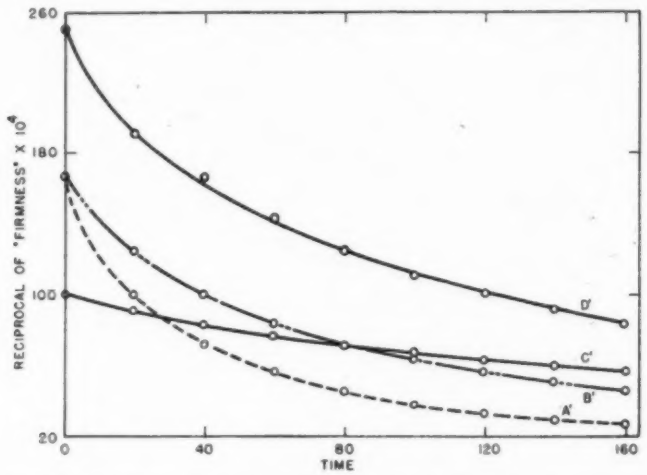

Fig. 5. Ideal "softness" data or the reciprocal of "firmness" of Fig. 4. General equation for the curves is: $S = 10^4/y = 10^4/(2mt + b)$, where S = reciprocal of firmness, t = time, $m_{A'} = 1$, $m_{B'} = 0.5$, $m_{C'} = 0.25$, $m_{D'} = 0.25$, and $b_{A'} = 60$, $b_{B'} = 60$, $b_{C'} = 100$, and $b_{D'} = 40$.

of breads C and D might be evaluated quite differently when "softness" is plotted rather than "firmness." The differences in the original "firmness" of the bread results in a variation in the rate of change in the slope of the "softness" curves which many would erroneously interpret as a reflection of differences in the rate of staling of the two breads. In contrast, the widely divergent "firmness" curves A and B indicate a wide difference in the staling rate of these two breads; whereas the "softness" curves A′ and B′ (Fig. 5) might lead one by superficial inspection and erroneous reasoning, to conclude that A′ and B′ are staling at essentially the same rate and that bread B′ is merely softer than A′. Closer inspection and calculation of the slopes at successive times, reveals that bread A′ apparently stales at

a greater rate than bread B′ between 0 and 40 time units; thereafter, the reverse is the case.

The apparent parallel nature of the "softness" curves A′ and B′ has been the chief misleading factor in interpreting these data and has led to the general but fallacious conclusion that few, if any, adjuncts have any appreciable effect on the rate of staling. Actually all "softness" curves must tend to approach one another gradually as they become asymptotic to a limiting horizontal axis. This asymptotic region of "softness" curves does not represent a true staling limit as has been inferred in the past but rather it is an artificial limit which depends on several factors in addition to the staling rate. This fact appears to explain why Cathcart (6) and others, have objected to crumb "softness" (as well as swelling power and soluble starch values which also decrease with time) as measures of staling, since the greatest changes occur within the first several hours after baking or at a time when bread is still considered fresh by human judges. As indicated by a comparison of curves C and D with C′ and D′ the initial "softness" reading greatly effects the shape of "softness" curves and such curves may lead one erroneously to think that maximum staleness has been approached merely because the curves level off rather sharply as they tend to become asymptotic. The slopes of "firmness" curves, on the other hand, are not affected by such artificial limits, but depend entirely on the rate of change of "firmness" readings.

The relative complexity of "softness" data, as compared with "firmness" values may be illustrated more precisely by comparing the first derivatives, (or slopes) of the curves shown in Figs. 4 and 5. In the case of the "firmness" data for which the general equation is $y = 2mt + b$, the slope is $dy/dt = 2m$, where b is the initial "firmness" reading, m the slope, t the time and 2 = "y" scale adjustment. In sharp contrast, the slope of the "softness" curves (Fig. 5) having the general equation $S = 10^4/y = 10^4/(2mt + b)$, is constantly changing and is represented by the relatively complex first derivative:

$$\frac{dS}{dt} = -\frac{10^4 \times 2m}{(2mt + b)^2}.$$

It is readily seen that the slope of the "softness" curve at any given time is dependent on the initial "softness" reading $10^4/b$, as well as on t and m. The slopes of "softness" curves are so complicated that one cannot be compared directly with another even with appropriate mathematical treatment. In accordance with common scientific practice, it is desirable, whenever possible, to express analytical data in a manner which leads to the most nearly linear relationship.

The preceding discussion is based on hypothetical ideal "firmness" curves, the equations for which were known; but the advantage of utilizing crumb "firmness" rather than crumb "softness" can be shown readily with data from the literature. As an example the crumb "softness" values obtained by Platt (19) are shown in Fig. 6, and the reciprocals of these values are plotted in Fig. 7. The "softness" data yield the well-known crumb compressibility curve, approximating that of an equilateral hyperbola, while the reciprocal values yield a much more nearly linear relationship; indeed considering the errors

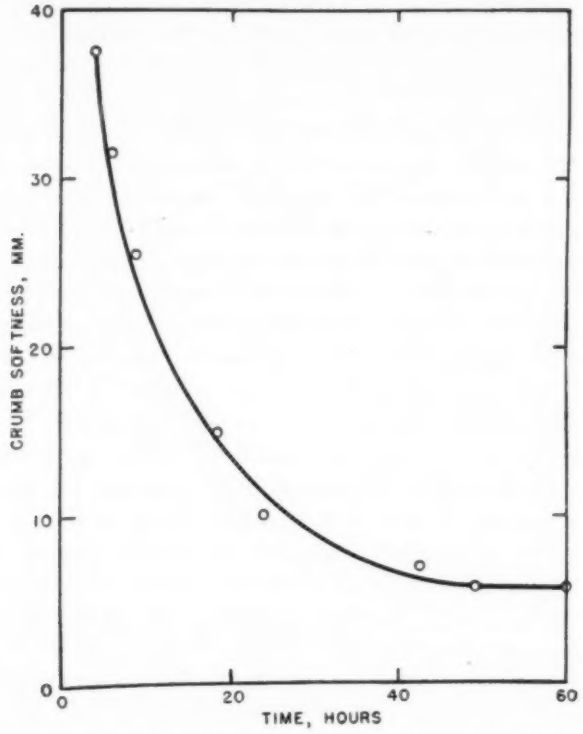

Fig. 6. Change in crumb softness of bread crumb with time. Data of Platt (19).

involved in this technique, the actual relation may be perfectly linear. Similar results were obtained upon the corresponding treatment of the compressibility data of Noznick, Merritt, and Geddes (18).

Also, direct determinations of crumb "firmness" by Crossland and Favor (10) using the Baker Compressimeter have given approximately linear curves when the force readings were plotted against a series of staling times up to 90 hours. Similar results have been consistently obtained by the present authors. Such data were the basis for the assumption in the foregoing theoretical treatment that "firmness"

data showed a linear change with time. It may be concluded that the forces required to yield a standard compression may be utilized directly to indicate differences in the crumb "firmness" of various breads at any time and also as a time index of the rate at which the bread becomes hard over the testing period normally employed. It is not, of course, implied that the crumb "firmness" change would continue to be linear over extended storage periods. In practice, interest in the rate of change is normally confined to the first few days.

It is clear that the conclusions which have been drawn in the literature from "softness" curves which represent (approximately)

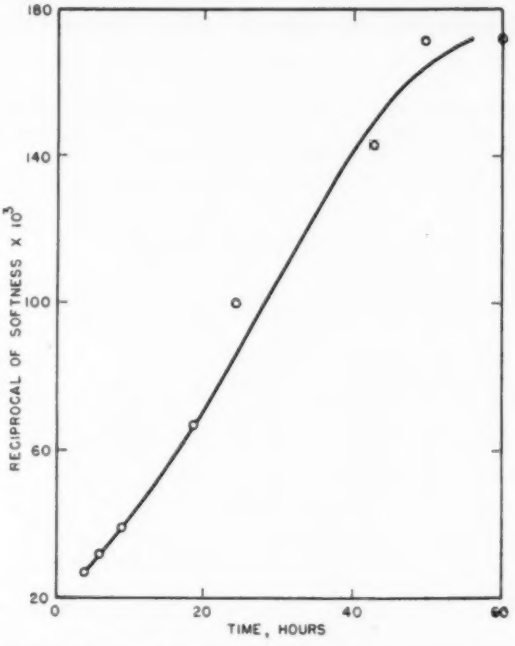

Fig. 7. Change in the reciprocal of crumb softness values (computed from data of Platt shown in Fig. 6) with time.

equilateral hyperbolae may well be erroneous. Similarly, other methods of measuring changes occuring during staling, the results of which markedly decrease with time and which yield similar curves, may also have been subject to erroneous interpretation. The Farinograph consistency data of Freilich (12) for example yield such curves, and the reciprocals show an approximately linear relation with time.

The authors regret that they were unaware of this anomaly when they reviewed the bread staling literature (14), as some of the conclusions may well require revision including those in papers with which one of us (W. F. Geddes) has been associated. A completely contrary

view of the relative merits of the two methods of expressing compressibility data has been recently expressed by Bradley (5).

Stress-Strain Relationships

Many series of force—deformation curves have been obtained in this laboratory for commercial white bread, army canned bread and laboratory-baked bread for various treatments and staling times but a few typical examples will serve to illustrate the general nature of the results. The maximum deformation which can be read with the Baker compressimeter, employed in this laboratory, is 4.0 mm. and it is possible to obtain consecutive deformation readings over a much wider range of forces in the case of canned bread than of ordinary

TABLE III

STATISTICAL CONSTANTS FOR DEFORMATION VALUES OBTAINED BY APPLYING VARIOUS LOADS TO CANNED BREAD DURING STORAGE AT ROOM TEMPERATURE[1]

Load	Mean[2] deformation	Variance		F for between times[2]	Standard error (single determination)	
		Between times[2]	Within times		Absolute	Per cent of mean
g.	mm.				mm.	
40	0.218	0.029	0.006	4.6	0.078	36.0
80	0.379	0.124	0.016	7.5	0.128	33.9
120	0.527	0.370	0.032	11.5	0.180	34.1
160	0.708	0.820	0.078	10.5	0.280	39.5
200	0.882	1.918	0.141	13.6	0.376	42.6
240	1.116	3.706	0.221	16.8	0.470	42.1
280	1.415	7.825	0.342	22.7	0.587	41.5
320	1.662	13.275	0.516	25.7	0.718	43.2

[1] Fourteen slices were read at ages of 0.92, 1.9, 4.6, and 9.75 days respectively.
[2] For staling times of 0.92 to 9.75 days inclusive.
[3] All values are highly significant.

white bread; the canned bread is more dense and contains less moisture so that it is firmer than ordinary loaf bread. The bread made according to the current army formula was sliced 0.5 inch thick, from which one-inch squares were cut and stored in air-tight containers. Deformation values were read on 14 replicate slices at loads from 40 to 320 grams in 40 gram increments for staling times of approximately 1.5 hours, 1, 2, 5, and 10 days. With 1.5-hour-old bread, it was not possible to obtain deformation readings above a 160-gram load. The mean deformation data for each load over all staling times are recorded in Table III together with a summary of a variance analysis of the individual data and the standard errors. The mean deformation values for each load and staling period are plotted in Fig. 8 with the exception of the data for 9.8 days, the curve for which practically

coincided with that for 4.6 days. The differentiation between the deformation values for the various staling times is increased by increasing the load; the *F* values for the differences in deformation due to staling time increase from 4.6 to 25.7 as the loads are increased from 40 g. to 320 g.

The experimental errors involved in reading deformation values are extremely high in comparison with other analytical determinations employed by the cereal chemist, which emphasizes the importance of employing a large number of replicates and carrying out statistical analyses before drawing conclusions concerning the influence of the variables under study. Part of the experimental error is due to variations in the density of the crumb from the top to the bottom of

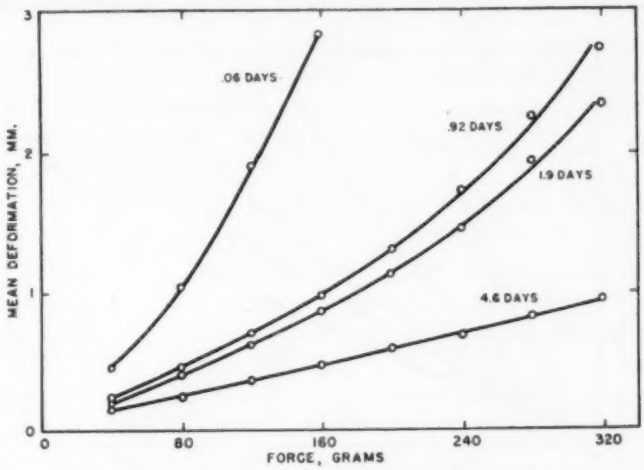

Fig. 8. Mean deformation values for army type canned white bread plotted against forces for various staling times. (Each point is a mean of 14 individual determinations, that is, 14 test slices.)

the can; in the majority of instances the crumb was softest at the center and firmest at the top.

The curves in Fig. 8 indicate that the relation between deformation and load varies for crumb of different ages. A statistical analysis revealed that for the 1.5-hour-old bread, the relation was curvilinear for even small loads. As the crumb aged and became less compressible the relation became more linear up to higher and higher loads. For the bread 0.92 days old the curve was found to be linear between loads of 40 and 160 g., for bread 1.9 days old it was linear between loads of 40 and 200 g., whereas for the bread 4.6 and 9.75 days old, the curves were linear between all loads employed. However, the maximum deformations to which the oldest breads were subjected was less than one mm. and did not approach those for the fresher samples, due to the fact that a load of 320 g. was the maximum for the instrument.

The choice of the force, or deformation, value for following bread staling will obviously influence the apparent staling rate since they will affect the extent to which the results will deviate from Hooke's law; the comparisons will include some values which lie on the approximately linear portion of the stress-strain curve for bread of a given age; at other staling periods the recorded values will represent data from the curvilinear portion.

A similar study was carried out with commercial white bread made without the addition of commercial emulsifiers or softening agents, employing a larger pressure plate and different slice thicknesses in an effort to improve the replicability of the test. Fifty commercial 1.5 lb. loaves were obtained from the same dough baked in a travelling oven. When cool, the bread was sliced into ½, ⅞, and 1¼ inch slices,

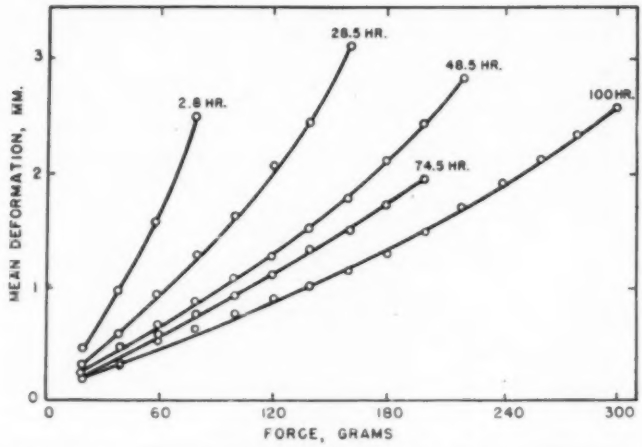

Fig. 9. Force-deformation curves for commercial white bread. Force vs. mean deformation on 1¼" slices.

cut into squares 2¾ × 2¾ inches and then placed in random fashion in air-tight cans at room temperature.

The first readings were obtained at approximately two hours after removal from the oven, and subsequent determinations were made at 24-hour intervals up to and including 100 hours. Fifteen slices were measured for each slice thickness, at each storage period. On each slice a series of deformation readings were obtained at successive 20-gram increments. The curves obtained with slices 1¼ inch thick shown in Fig. 9 are typical of the series. In all cases there was a curvilinear relation between load and deformation particularly for the freshest breads. The standard errors for comparable deformation values were considerably lower than those obtained with canned bread but they were still very high; the standard errors of single determina-

tions expressed in per cent of the mean varied from 11.0 to 33.9% for the one-half inch slices. As the slice thickness was increased the per cent error also increased and the greater area of the compression plate in this series did not reduce the error to desirable levels. With laboratory baked bread, the force-deformation relations were more curvilinear at corresponding staling times than were found for the commercial bread. It therefore appears that if the deformation exceeds a very low value, the force-deformation ratios are not constant.

It should be emphasized that the preceding force-deformation data were all obtained by reading the deformation obtained under standard force increments and that the error involved in attempting to read variable deformation is large.

The error is markedly lower, however, when force-deformation data are obtained by reading force (instead of deformation) required to give a series of arbitrary deformation values. By this method, the standard errors of a single determination (expressed as a per cent of the mean) have been found to be in the order of 9 to 10% for 2.5 mm. deformations.

Apparently, one can read variable force values with a considerably greater degree of accuracy than when attempting to estimate the small changes on the deformation scale of the Baker compressimeter. This fact represents another valid reason for determining "firmness" rather than "softness" when evaluating staling with the Baker compressimeter.

The force-deformation data obtained by reading variable force values, however, likewise yield curvilinear relationships, exemplified in Fig. 10.

The question naturally arises as to whether bread obeys Hooke's law under the conditions employed. That is, whether strain is proportioned to the stress. Since strain equals the deformation divided by the original crumb thickness (a constant), it is not necessary to transform the deformation readings in order to test the relationship. However, stress equals the force divided by the effective cross sectional area of the test object and since this effective area of force changes during deformation, it would be desirable to calculate the stress values.

In the present study, crumb slices slightly larger than the area of the plunger were used, and it was apparent from observation of the bending of the crumb surrounding the plunger, that the effective area under load was increasing with increments of force.[6] Dividing the force readings of Figs. 8, 9, 10 by increasing area values in order to obtain stress readings would yield stress-strain curves which would

[6] The change in cross-sectional area under compression represents another reason for employing a "firmness" method for evaluating staling changes, since all comparisons are made on bread at the same standard compression and hence same effective cross-sectional area.

tend to be more curvilinear. Consequently, without attempting to evaluate the magnitude of the increase in effective area, it can be stated that the bread did not strictly obey Hooke's law.

It must be concluded that, under the conditions of these experiments, bread crumb does not behave as a perfectly elastic body but as a plasto-elastic solid. In the case of fresh crumb, plastic flow is an appreciable factor in the compression which is observed, but as the bread stales and becomes rigid, presumably by the establishment of a three-dimensional structure through the formation of cross linkages, the possibilities for plastic flow become more and more restricted and the behavior of the bread closely approaches that of a pure elastic body. It would therefore be expected that the elastic regain of stale

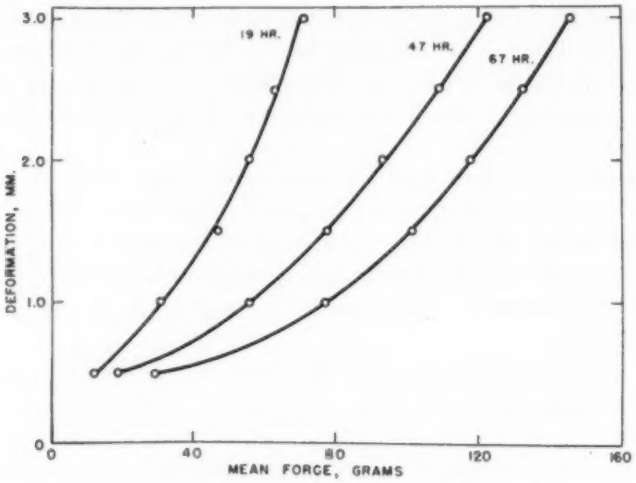

Fig. 10. Mean force values for laboratory baked white bread plotted against deformation for various staling times. Each point is a mean of 10 individual determinations.

bread would be greater than that of fresh bread; moreover, any treatment which would increase the softness would tend to cause the bread to deviate more from Hooke's law and, by increasing the possibility for plastic flow, would yield bread exhibiting poor elastic recovery from the application of stress.

While the failure of bread strictly to follow Hooke's law is not surprising because of its complex nature, it is a complicating factor in the use of the compressibility technique for following the changes in crumb hardness. The apparent rate of change will be influenced by the experimental conditions which are employed, such as the slice thickness and the standard deformation which is selected for firmness readings. Moreover, the results cannot be subjected to precise

mathematical treatment; the reciprocals of crumb softness data do not correspond exactly with crumb "firmness" readings, and the results cannot be expressed in absolute units.

Farinograph Method

The mathematical difficulties involved in attempting to evaluate the curvilinear Farinograph data have been pointed out in previous sections. In some instances these data may be converted to linear data, by appropriate mathematical manipulation; but there still remains considerable doubt as to what relation, if any, exists between the Farinograph data and bread staling. Several laboratory experiments have served to strengthen this doubt as may be seen from the following examples of studies on 40% starch pastes.

Maximum Farinograph consistency values obtained with 40% wheat starch pastes during storage decrease with time just as in the case of bread crumb. On the other hand, consistency values for 40% waxy starch pastes (corn and sorghum) increase with time, as though becoming "fresher," while over the same time interval these initially soft pastes set to form hard, almost cartilaginous gels. Typical results follow:

Age of paste	Maximum Farinograph consistency	
	40% wheat starch paste	40% waxy sorghum starch paste
hr.	*B.U.*	*B.U.*
4	360	325
24	360	425
48	345	440
70	260	450
95	122	—

These results indicate that the Farinograph and "firmness" methods may give widely different results. In addition, it could be inferred that the physical changes in bread may involve more than changes solely in the amylopectin fraction of starch.

When polyoxyethylene stearate was added to a 40% wheat starch slurry in an amount equivalent to 0.5% of the total solids and the mixture gelatinized, the fresh paste was whiter, less sticky, and more crumbly than the control. Upon mixing the treated paste in the Farinograph, a coherent mixture was not formed; the particles merely rode on the blades without offering appreciable resistance to the mixing force with the result that a low or "stale" consistency reading was obtained which was not representative of the true consistency nor the age of the paste. After storing the treated paste for several weeks, it was still white and soft to the touch.

Similar anomalous results were obtained by Freilich (12) upon comparing the Farinograph and compressibility methods on bread containing this adjunct.

Measurement of Swelling Power

During staling, the ability of bread crumb to absorb water decreases. This so-called "swelling-power" change has been followed in the laboratory by several procedures in which crumb is first pulverized in water and the swelling power then determined by measurement of the crumb volume after sedimentation (16) or centrifuging (7); or by the increase in weight of crumb sediment after centrifugation of the slurry (22). Measurement of capillary flow of a thin pulverized crumb slurry has also been used (15).

Here again with this method, one apparently must exercise care in interpretation of results, as it was found that swelling power values for 40% waxy sorghum starch pastes *increased* with time instead of decreasing. Just as in the case of the Farinograph consistency values, such an increase would give the false impression that the paste was becoming "fresher," while over the same time interval, the stored pastes became very firm. Typical results are summarized below:

Age of paste	Swelling power [1]	
	40% wheat starch paste	40% waxy sorghum starch paste
hr.		
2	4.77	2.87
24	4.09	2.99
48	4.06	3.24

[1] Swelling power = $\frac{\text{weight centrifuged crumb sediment}}{\text{original weight crumb sample (dry basis)}}$.
All values are means of duplicate determinations.

In applying such sedimentation methods to staling studies careful consideration must be given to the various factors which may be involved. For example, in applying this method to a study of the effects of chemical adjuncts, the sedimentation height of the crumb particles will depend upon their sedimentation velocity which in turn is influenced by several variables embodied in Stokes' Law of falling bodies.[7] The adjunct might be adsorbed on the crumb particles and influence their specific gravity or crumb particle radius, or change the

[7] Stokes' Law of falling bodies:

$$V = \frac{2}{9} \frac{(D - d)}{\eta} G r^2,$$

where V = velocity of fall;
D = the specific gravity of the falling particle;
d = the specific gravity of the medium through which the fall takes place;
η = the viscosity of the medium;
G = the gravity constant;
r = the radius of the particle.

viscosity of the dispersion medium. Hence it is difficult to interpret crumb sedimentation data, particularly when obtained on breads made by different formulas.

Measurement of Soluble Starch

The amount of soluble starch that can be leached from the crumb with water decreases with aging of the loaf. This method (16, 22) consists of precipitation by alcohol, recovery, and drying of this starch, but is so time consuming that it is not often used. Here again, one must be cautious in drawing conclusions when comparing treated with control breads. If, for example, an anti-staling agent were able to form a water-insoluble complex with the soluble starch, a low, or "stale" soluble-starch value would be obtained since the usual amount of soluble starch would not be extracted from the crumb. However, this could actually mean in this case that staling is being partially inhibited because the soluble starch would no longer be free to aid in building up an immobile three-dimensional gel structure.

Reference has already been made to the fact that Katz (16) found that the swelling power and soluble starch methods gave widely different results when used to evaluate the effect of acetaldehyde on bread staling.

Measurement of Crumbliness

The crumbliness values increase with time and would appear to be of interest in relation to consumer acceptance. It seems probable that a certain level of crumbliness would be associated with tenderness and ease of mastication whereas excessive crumbliness would be undesirable since the bread would not slice well. On the basis of this concept, a crumbliness method would have to be "calibrated" by consumer acceptance tests to determine the optimum crumbliness range before one would be in a position to state whether a change in crumbliness due to different formulas or treatments is desirable or undesirable. This reasoning also applies, of course, to other methods which have been used as indices of staling. This method is of somewhat limited value because of the difficulty in obtaining reproducible results.

Discussion

These studies serve to indicate the difficulties which are involved in following the staling of bread crumb by the laboratory methods which are in common use. Some of the contrary conclusions in the literature are attributable to differences in defining and measuring staling.

Staling, as it is applied to bread, is a generic term covering a number of ill-defined changes that occur in bread as it ages. Consumers judge

staleness by direct perception, which provides a subjective estimate that probably represents an unconscious integration of many factors. As bread ages certain phenomena are readily apparent: the flavor and aroma change (probably through loss of volatile constituents), the crumb becomes firmer, crumbles more readily and feels harsher to the tongue. These gross changes are doubtless the result of complex physico-chemical reactions within the loaf. These gross changes and the various underlying reactions, as well as other physical or chemical phenomena which contribute to the subjective estimate comprise the process commonly called "staling."

Investigators who are engaged in devising methods for measuring certain properties of bread and in determining how these properties change as bread ages tend to assume that each method measures "the rate of staling" and to argue the relative merits of different methods from this viewpoint. It seems more reasonable to suppose that any one physical or chemical method can measure, at best, only a few of the factors involved in the subjective integrated assessment of staling. Each method can reveal only part of the story. Moreover, since there is no *a priori* reason for supposing that the various processes involved in staling take place at the same rate, there is no reason to expect that methods that measure different properties will give identical data for rates of change in the crumb.

It appears that the comparative merits of various objective measures of bread staling can only be determined adequately through the use of organoleptic tests made by a panel of experts under a strict statistical control. The panel would hardly be able to make quantitative estimates of staleness, that is, to judge whether one sample was two or three times as stale as another; but given a series of bread samples, it should be possible to rank them at least roughly in order of "staleness." The physical or chemical measurements that place the same series in most nearly the same rank order as the panel may then be said to be the best objective method of measuring staleness.

Until a study of this nature is made, it seems that methods now in use must be accepted for what they actually are, namely, methods of measuring the change in some property or properties of bread with time. That some properties, such as crumb firmness and crumbliness, are related to staling is a reasonable inference but to suppose that any one method actually served as a measure of the whole process seems unjustified on the basis of our present knowledge.

Acknowledgments

The authors are indebted to P. P. Noznick who developed the modified empirical crumbliness test and to R. Koch and R. Stenberg for technical assistance in carrying out the comparative study of various methods for following the staling process. They are also grateful for the helpful suggestions of J. A. Anderson, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, in preparing the manuscript.

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SOME PHYSICAL VARIABLES AFFECTING THE GASEOUS BLEACHING OF FLOUR [1]

W. W. DODGE and MAX MILNER

ABSTRACT

A number of physical variables, including time of gaseous treatment, agitator loading, moisture content of the flour, temperature, and time of reaction, influence the efficiency of flour bleaching with nitrogen trichloride, as determined from the carotene color value of flours treated in a laboratory-scale bleacher.

Bleaching efficiency decreased with extent of treatment, and pigment removal beyond 0.65 ppm. could not be obtained even at extremely high levels of treatment with the flour used in this study. Nitrogen trichloride reacted with flour at a very rapid rate. Duration of gas treatment, irrespective of amount of gas, was a major variable affecting efficiency, and this factor in turn depended on agitator loading. The mixing of bleached and unbleached flour conferred no bleaching on the untreated flour.

Temperature was inversely related to bleaching efficiency, indicating the possibility that adsorption, which is influenced negatively by temperature, limits the chemical action of nitrogen trichloride on flour pigments. At the same rate of application more pigment was destroyed at low temperatures; at higher temperatures more of the gas apparently reacted with other flour constituents. Increased moisture content of flour similarly caused a decrease in bleaching efficiency.

A simple test of efficiency of commercial bleaching equipment, in terms of pigment removal obtainable with a laboratory bleacher, is outlined.

The influence of a number of physical variables which may affect the commercial flour bleaching process was investigated with the objective of originating technics to secure optimum efficiency with gases which are now widely used for both bleaching and maturing. These factors, which included duration of gas application, temperature of flour, flour moisture content, agitator loading, and velocity of reaction, were studied with laboratory-scale equipment. The bleaching gas used was nitrogen trichloride. That some of these variables may have a major influence on bleaching efficiency was apparently overlooked in previous studies of gaseous flour bleaching which have stressed either the purely chemical aspects of the subject (2, 8, 9) or the comparative efficiency of various bleaching and maturing gases when applied under pilot-scale and standard mill conditions (3, 4, 5, 6, 7).

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Presented at the Annual Meeting May, 1949. Contribution No. 163, Department of Milling Industry, Kansas Agricultural Experiment Station, Manhattan, Kansas. This research was supported by a grant from the Wallace and Tiernan Company, Inc., Newark, New Jersey.
A portion of a thesis presented by W. W. Dodge as partial fulfillment of the requirements for the degree of Master of Science in Milling Industry at Kansas State College.

Materials and Methods

The major portion of this study involved the use of a motor-driven laboratory flour agitator similar in design to that manufactured by the Wallace and Tiernan Company except that it was octagonal in shape and contained a system of deflecting paddles intended to increase the mixing efficiency. The agitator was maintained in a thermostatically controlled constant temperature cabinet and was driven at 29 rpm. Nitrogen trichloride was generated by forcing a measured quantity of chlorine gas into a solution of ammonium chloride contained in a reaction vessel. The nitrogen trichloride generated by the interaction of these reagents is aerated from the mixture into the flour agitator. The bleaching efficiency expressed as percentage of pigment removal was determined by the water-saturated N-butyl alcohol extraction method for flour pigments as outlined in *Cereal Laboratory Methods* (1), using an Evelyn photoelectric colorimeter. This determination was carried out 24 hours after the flours were bleached. The response of the standard flour to nitrogen trichloride treatment and the optimum treatment level were determined by a baking test carried out by the straight dough pup-loaf method described in *Cereal Laboratory Methods*, using 6% sugar, 4% milk, 1.5% salt, and 3% shortening, on a 14% moisture basis for the flour.

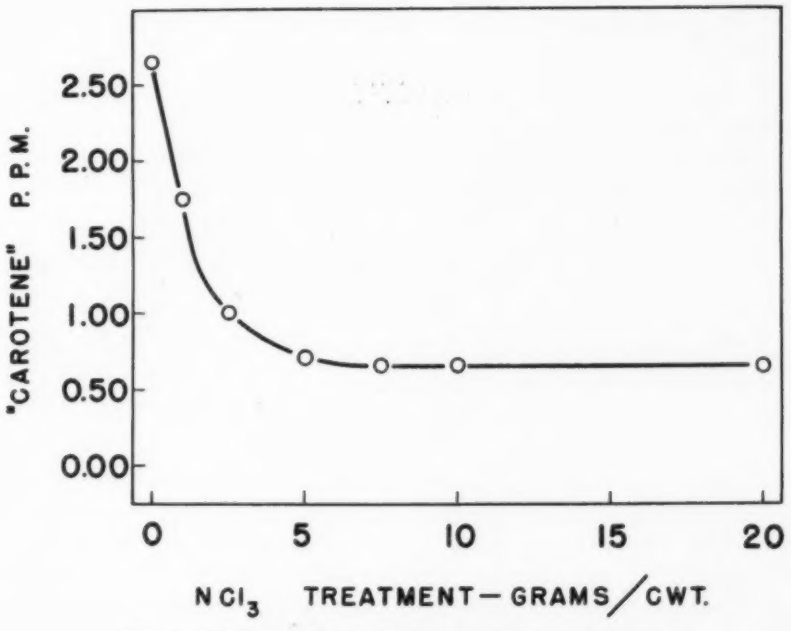

Fig. 1. Influence of dosage of reagent on pigment concentration.

The flour used throughout this study was a straight grade milled to 68% extraction from a typical commercial mill mix of southwestern hard red winter wheats, and was representative of the quality of the 1948 crop. It contained 11.7% protein, 14% moisture basis.

Results

Influence of Dosage on Extent of Bleaching. The influence of the dosage of nitrogen trichloride on bleaching efficiency at room temperature in terms of removal of pigment was investigated by analyzing for carotene flour samples treated at the following dosages (g./cwt.): 1.0, 2.5, 5.0, 7.5, 10.0, and 20.0. The data obtained in this experiment relating residual pigment concentration to bleaching rate are shown in Fig. 1. Reference to Fig. 1 indicates that the pigment removal from this flour is very great with treatments up to 2.5 g. of nitrogen trichloride per hundredweight, but is less rapid as the treatment is increased to levels as high as 7.5 g./cwt. No removal of pigment from this flour beyond 0.65 ppm. could be obtained with treatments higher than 7.5 g. This curve probably represents a normal pattern for all flours bleached with nitrogen trichloride and indicates that a residual pigment remains which is impervious to oxidation by this reagent. Normally, further bleaching is accomplished in mills by the use of benzoyl peroxide.

Influence of Duration of Gas Treatment and Agitator Load. The relationship between the time of addition of a fixed charge of gas to bleaching efficiency and the influence of variable loading on the time of gas application required for optimum efficiency was investigated. Nitrogen trichloride was introduced in the amount of 2.5 g./cwt. of flour in a bleaching program in which 2-, 4-, and 6-pound samples were treated over intervals from 25 seconds to 6 minutes as follows:

2-lb. samples		4-lb. samples		6-lb. samples	
Min.	*Sec.*	*Min.*	*Sec.*	*Min.*	*Sec.*
0	25	1	17	2	0
0	35	1	45	2	45
0	45	2	6	3	0
2	9	3	6	3	34
2	30	3	40	4	0
3	0	4	25	5	25
4	0	5	0	6	0

The 2.5 g. level of treatment was selected by a previous baking trial as the one which gave nearly optimum color removal with maximum improvement of baking properties. The results presented in Fig. 2 indicate that at light loading (2 lb.) maximum bleaching efficiency (61% pigment removal) could be obtained when the gas was introduced over an interval of 45 seconds, whereas with shorter time intervals

of gas introduction (25 and 35 seconds) a drastic decrease in bleaching efficiency occurred. Extension of the time of gas application beyond 45 seconds in this case caused no further increase in efficiency. A similar trend was noted with heavier loading of the agitator. The 4-lb. loading reached a maximum degree of color removal only after 2 minutes and 15 seconds of gas application, whereas the 6-lb. load required 4 minutes and 30 seconds. It is significant that the identical quantity of gas introduced over a short period did not produce as great

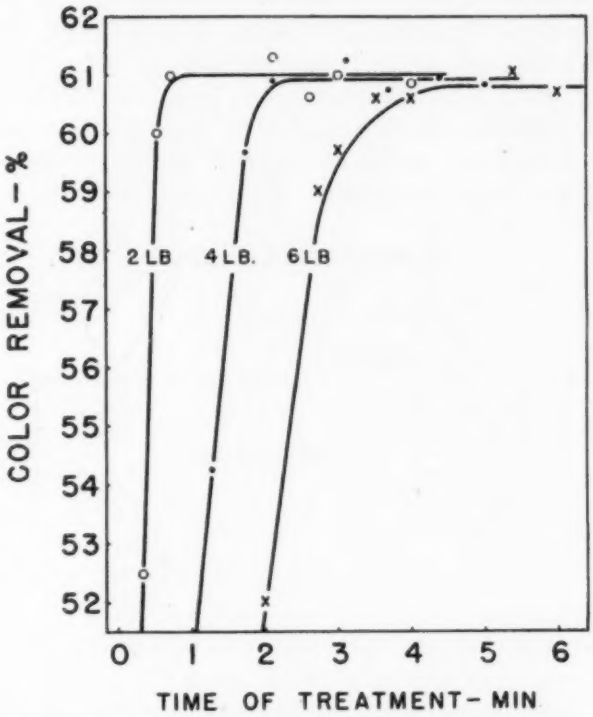

Fig. 2. Influence of time of treatment with nitrogen trichloride and agitator loading on color removal.

color removal as could be realized when the time of treatment was extended. These data indicate that a decrease rather than an increase in efficiency is obtained when the agitator load is increased with respect to time of gas treatment.

Estimate of Velocity of Reaction of Nitrogen Trichloride with Flour. To obtain an indication of the rate of reaction of nitrogen trichloride, 150 g. of flour were packed into a cylindrical glass tube between glass-wool plugs and perforated rubber stoppers as shown in Fig. 3. A quantity of nitrogen trichloride mixed with air to a volume of 2400 ml., sufficient to treat 150 g. of flour at the rate of 60 g./cwt., was forced

through the column of flour for 10 minutes at a pressure of 10 lb. per square inch.

Two distinct zones of color appeared in the tube of flour. A first zone of flour, approximately ¼ inch in thickness, appeared to be very white and showed a pink coloration at the first surface of contact of the gas with the flour. The balance of the flour in the column, however, appeared to be unbleached and the boundary between the bleached and unbleached flour was clearly defined. These relationships were confirmed by careful separation of the flour in the different zones and analysis for pigment content. The bleached zone, ¼ inch thick which contained 10% of the total flour in the tube, apparently had retained 100% of the bleaching gas. In spite of this high retention of nitrogen trichloride this bleached flour showed only a 60% removal of pigments, which is comparable to that obtained with normal commercial treatment (ca. 2 g./cwt.). The extreme rapidity of the action

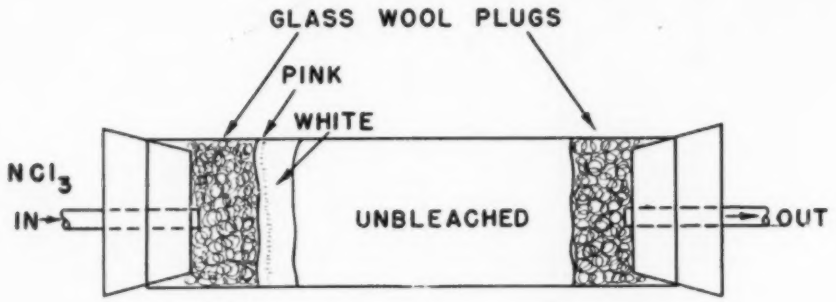

Fig. 3. Diagrammatic view of flour column in glass tube after treatment with nitrogen trichloride.

of nitrogen trichloride on flour is indicated by this experiment and the speed of the reaction can be calculated from the results.

Packed flour has about 52% of free air space, leaving 48% actually occupied by flour particles. Thus the cross-sectional area of the tube, which is 2.036 square inches, would be made up of $2.036 \times \frac{48}{100}$, or 0.975 square inches of flour, leaving 1.06 square inches of intergranular space for gas flow. The volume of gas used was 2400 ml. or 146.5 cubic inches and the time of passage was 10 minutes. Thus the rate of flow through the flour cross section was $\frac{146.5}{1.06 \times 10}$, or 13.8 inches per minute. Assuming that bleaching was complete in a zone 0.25 inch thick, the gas would pass through the bleached layer in only $\frac{13.8}{60 \times 0.25}$ or 0.92 second.

This calculation shows that any portion of the gas reacted with the first 10% of the flour column within one second from the time of initial contact.

Influence of Blending Bleached and Unbleached Flours. It has been shown that nitrogen trichloride reacts very rapidly with flour and that mixing efficiency decreases sharply with overloading. Under certain conditions it is possible, therefore, that the gas intended to treat a certain quantity of flour may react with only a portion of the total amount, with the result that part of the flour will remain either partially bleached or even entirely unbleached. Under practical conditions this could occur through the application of gas over too short a period

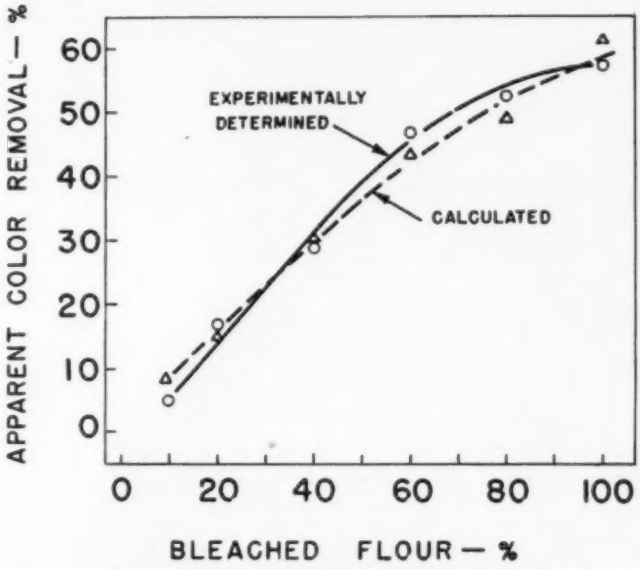

Fig. 4. Influence of blending bleached and unbleached flour on apparent color removal with nitrogen trichloride.

to effect uniform distribution of the bleaching gas, with the result that the total bleach is not equally distributed throughout all the particles of flour. It was the purpose of the following experiment to determine the influence of such conditions on bleaching efficiency.

Subsamples of a flour were treated with nitrogen trichloride to the following levels (g./cwt.): 25.00, 12.50, 6.25, 4.70, 3.13, and 2.50. Bleached lots were then blended with unbleached flour to yield samples all containing 2.5 g./cwt. of bleach and were analyzed for carotene by the usual method. The data obtained are presented in Fig. 4 together with the calculated values for the final pigment content, using the dilution factor of added flour. This curve followed that obtained experimentally very closely.

These results indicate clearly that overbleaching one portion of flour does not confer bleaching to unbleached flour mixed with it, and that the final pigment content is the weighted average of that of the two individual samples. In commercial practice one would probably be dealing with flour compounded of material underbleached to various degrees mixed with flour at various stages of overbleaching.

Effect of Temperature. To determine the effect of temperature on bleaching, the following experiment was carried out.

Identical 4-lb. samples of flour were sealed in glass bottles and brought to 7°C., 15°C., 26°C., 40°C., and 50°C. respectively in a controlled constant temperature cabinet. The various samples were

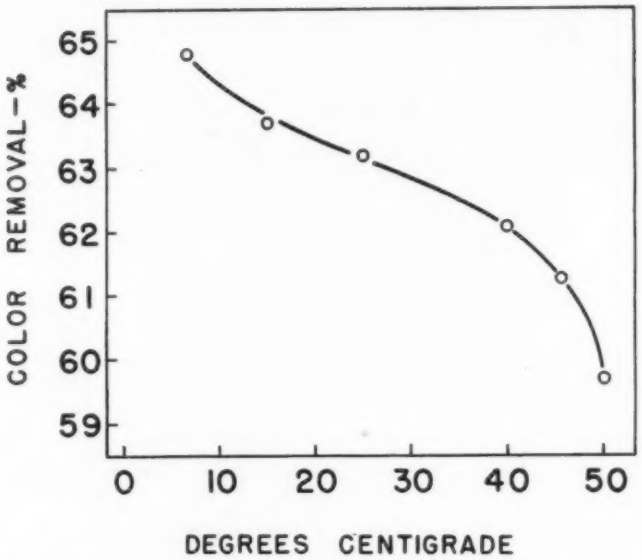

Fig. 5. Effect of temperature on bleaching efficiency with nitrogen trichloride.

bleached at these respective temperatures for 4 minutes at the standard rate of nitrogen-trichloride treatment (2.5 g./cwt.). The results, which are averages of duplicates, at each temperature are presented in Fig. 5.

The downward trend of the bleaching curve with increasing temperature was surprising since the rate of a purely chemical action can be expected to increase with an increase in temperature. Other explanations than purely chemical reaction phenomena must therefore be sought. Since adsorption has a negative temperature coefficient, it is highly probable that adsorption precedes chemical reaction in the bleaching of flour with nitrogen trichloride. It is to be noted, however, that regardless of the variation in efficiency of bleaching with tempera-

ture, the total amount of nitrogen trichloride applied to the flour was the same at all temperatures. This might indicate that as the rate of gaseous combination with flour is reduced by an increase in temperature, the less does the bleaching gas react with the pigment and the more do other flour components, particularly protein, enter into reaction with nitrogen trichloride. In other words, temperature, in determining the rate of adsorption, may affect the differential rate of reaction between nitrogen trichloride and various flour components.

The Effect of Temperature as Related to Time of Treatment. In the previous experiment, increasing temperature was found to decrease the

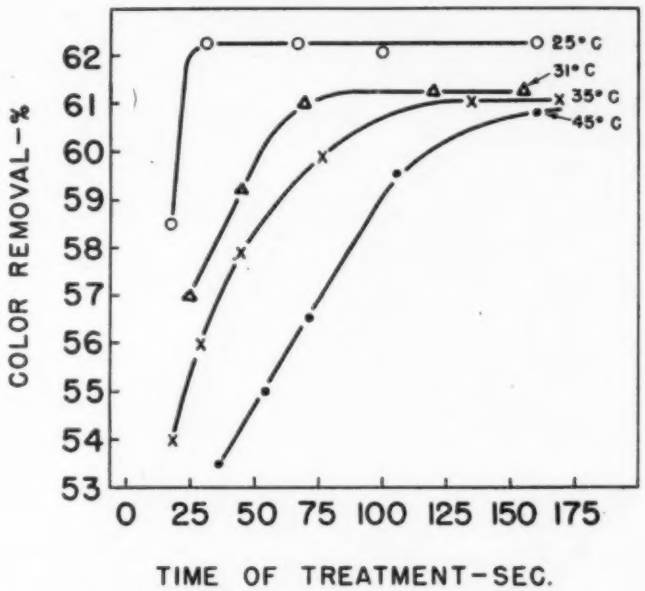

Fig. 6. Influence of temperature and time of treatment with nitrogen trichloride on color removal.

efficiency of bleaching when the gas was applied over a period of 4 minutes. It was of interest to ascertain whether increased efficiency at the higher temperatures might be obtained if the time of gas treatment were extended. For this experiment, flour was bleached over various time intervals at 25°C., 31°C., 35°C., and 45°C.

A representative experiment was as follows: Ten sealed bottles of flour, which had been maintained at 10°C., were placed in the constant temperature cabinet at 45°C. for about 16 hours before bleaching. Different intervals of treatment with nitrogen trichloride at the various temperatures were applied. All samples were bleached in duplicate and the average values are presented in Fig. 6.

At low temperatures, e.g., 25°C., maximum utilization of the gas is obtained in much shorter periods than at higher temperatures. As the temperature is increased, the time required to produce the maximum bleach is rapidly extended until at 45°C. the optimum bleach was reached only after 3 minutes, as compared to 0.5 minute at 25°C.

This experiment proves conclusively that temperature is inversely related to bleaching efficiency. As the temperature is increased, the

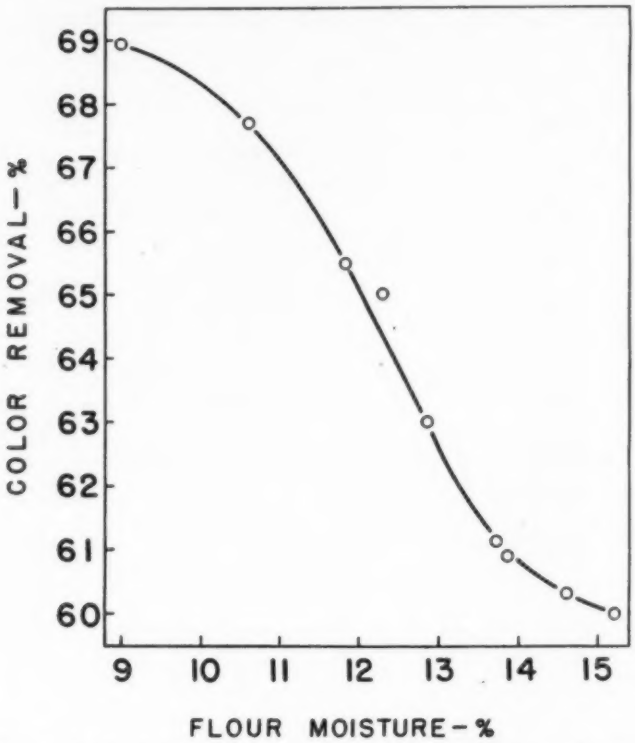

Fig. 7. Influence of flour moisture on color removal with nitrogen trichloride.

time required to obtain a maximum bleach is increased, while the efficiency with respect to color removal decreases as the temperature is increased.

Effect of Flour Moisture on Bleaching Efficiency. Moisture content is a major variable in flour processing, and any influence of this factor on bleaching efficiency with gases would be important to milling operations. A group of flour samples with moisture values ranging from 8.9 to 15.2% were bleached with nitrogen trichloride at the rate of 2.5 g./cwt. Bleaching time, temperature, and loading were held con-

stant in this experiment. Moisture content was determined by the air oven method as outlined in *Cereal Laboratory Methods*. The results of this study appear in Fig. 7.

These data do not bear out the widely held opinion in the milling industry that bleaching efficiency increases with moisture content, since exactly the reverse was found to be true. The present data indicate that a decrease in bleaching efficiency occurred throughout the moisture range investigated, namely 8.9 to 15.2%, and that the greatest downward inflection of the curve occurred in the region of 12% moisture. A possible explanation of this phenomenon is the low solubility of nitrogen trichloride in water. As the moisture content of

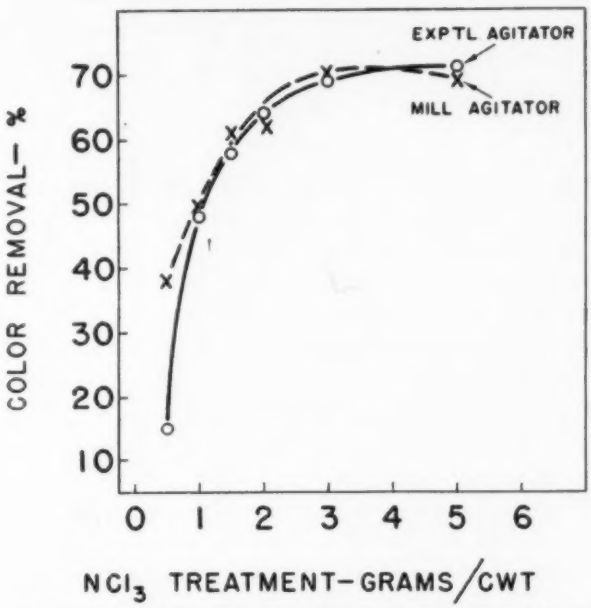

Fig. 8. Comparative efficiency of experimental agitator and commercial mill agitator.

a flour increases it may be expected that its affinity for nitrogen trichloride would decrease.

Relative Efficiency of Experimental Agitator and Commercial Scale Mill Agitator. Ferrari and co-workers (5) stated that pilot-scale bleaching is less efficient than bleaching on an experimental basis, and that the commercial bleaching process was even less efficient than that carried out on pilot scale. It was of interest, therefore, to compare the efficiency of the small box agitator with the commercial No. 1 Alsop agitator in the Kansas State College mill. The latter was operated at 135 rpm. and a load of 500 lb. per hour, which is the normal production rate of the mill and is very low for agitator equipment of this

size. Using commercial Agene generating and metering equipment, samples were bleached at the following rates (g./cwt.): 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0. An unbleached lot of this flour was taken for treatment in the experimental agitator at the same rates using a loading of 2 lb. and a time of gas treatment of 4 minutes. The percentage of color removal obtained in both studies is given in Fig. 8.

These curves show that no significant difference existed in the efficiency of bleaching between the mill and experimental agitator. This result differs from those obtained by Ferrari and co-workers (5) who found the experimental type to be more efficient. The high efficiency of the mill bleacher in the present study was due to the fact that it was very lightly loaded. In any event it is apparent that commercial equipment can be operated to yield optimum utilization of bleaching gas and that many of the variables affecting its efficiency still remain to be determined.

Discussion

These experiments indicate that simple physical variables which normally may be encountered in flour bleaching with gases may profoundly affect the efficiency of the process. The very rapid rate of reaction of nitrogen trichloride with flour must be taken into account in determining the time to be used for introducing a given amount of gas, in order to obtain optimum bleaching efficiency. The inverse relationship of bleaching efficiency to temperature and moisture content shown in the present studies has not been generally recognized and is of fundamental importance in any consideration of methods to improve the bleaching process. The results of these studies have emphasized the complex nature of the reaction of nitrogen trichloride with flour. Pigments, fats, and proteins all appear to be affected by nitrogen trichloride, and physical variables such as temperature, moisture, extent of treatment, and duration of treatment will influence the degree of reaction of the various components of the flour with the gas.

A few facts regarding gaseous bleaching, which hitherto have been generally assumed, have been demonstrated experimentally in this study. These include the fact that the mixing of bleached with unbleached flour confers no bleaching to the untreated portion. It also has been clearly shown that nitrogen trichloride treatment allows a residual pigment, which is unaffected by enormous concentrations of the gas, to remain in the flour. It was further shown that an increase of gas concentration with increased agitator loading does not compensate for the loss in mixing efficiency caused by the increased load. Application of these laboratory-scale results may enable commercial bleaching to approach the laboratory batch process in efficiency.

It appears desirable to express the efficiency of commercial bleaching with gases by a numerical value which would take into account the pigment content of the original unbleached flour as well as the maximum bleach which can be applied to the flour by the most efficient methods. The maximum bleach for a given gas level would be that secured with an efficient laboratory agitator. This value in ppm. of carotene would be obtained by treating the unbleached flour with the gas at the same rate at which it was bleached in the commercial-scale agitator. This test bleach must be conducted at the same temperature and moisture content as in the commercial agitator.

The calculation of the empirical efficiency rating requires the assumption that the color value of the commercially bleached flour is an average of two lots of flour mixed together, one bleached to the maxi-

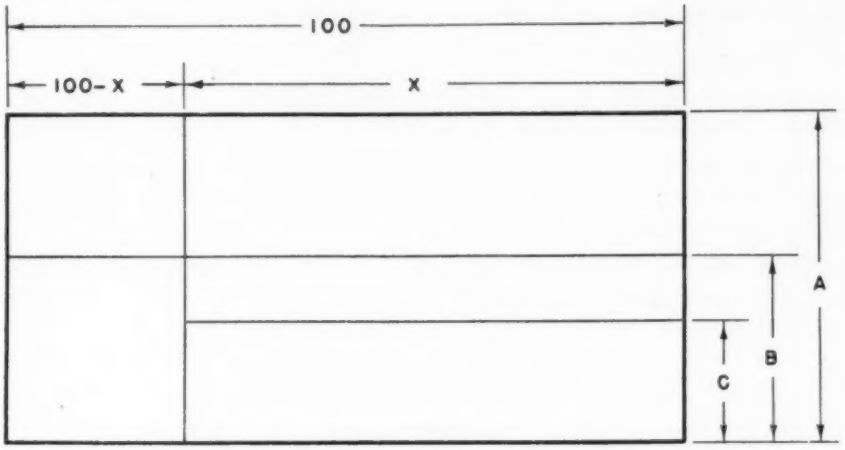

Fig. 9. Diagram for geometric development of equation for efficiency.

mum value obtainable in the laboratory bleacher, and the other entirely unbleached. The calculation of efficiency therefore involves not only flour pigment content in terms of ppm. carotene, but also the relative quantities of the two flours (bleached and unbleached) in the mixture. Pigment determinations on unbleached flour, commercially bleached flour, and laboratory bleached flour are the only analytical values which need to be secured.

A geometric development of an equation for efficiency may be made with the aid of a diagram, as shown in Fig. 9.

Let A equal the color content in ppm. of the unbleached flour.

Let B equal the color content in ppm. of the commercially bleached flour.

Let C equal the color content in ppm. of the laboratory bleached flour. In this case A is greater than B which is greater than C.

X equals the portion of the commercially bleached flour, expressed as a percentage, which contains the maximum bleach.

100 − X equals the percentage of unbleached flour in the commercially bleached sample.

Area due to commercially bleached flour equals 100B.

This area may be equated to the sum of the areas $CX + A(100 - X)$.

Equating these values:

$$100B = CX + A(100 - X).$$

Solving for X:

$$X = 100 \times \frac{(B - A)}{(C - A)}.$$

The term X can be visualized as that portion of the commercially bleached flour expressed in per cent which contains a bleach value equivalent to that obtainable with the laboratory bleacher and might well be called the agitator efficiency, or simply A.E.

Thus

$$\text{A.E.} = \frac{(B - A)}{(C - A)} \times 100.$$

As an example of the application of this equation, assume a practical case where the unbleached, commercially bleached, and laboratory bleached flours have color values of 2.5, 1.2, and 0.9 ppm. of carotene, respectively. The agitator efficiency, A.E., of the commercial equipment in the case of this example is

$$\frac{(1.2 - 2.5)}{(0.9 - 2.5)} \times 100 = 80\%.$$

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METHODS FOR THE LABORATORY-SCALE PRODUCTION OF CHLORINE DIOXIDE AND THE TREATMENT OF FLOUR [1]

HUGH K. PARKER [2] and KARL L. FORTMANN [2]

ABSTRACT

Two practical methods for the small-scale generation of chlorine dioxide have been developed. In the first method, pure chlorine dioxide is measured volumetrically, followed by dilution with air and its subsequent application to the flour. In the second method, chlorine dioxide is prepared in aqueous solution (by the reaction of acetic anhydride with sodium chlorite); the gas is then aerated off and directly applied to flour.

Since chlorine dioxide has been approved as a flour maturing and bleaching agent, a safe, practical, simple, and reliable method for generating chlorine dioxide and for treating flour on a laboratory-scale is needed. Such a method should make most efficient use of existing laboratory equipment and facilities.

Hutchinson and Derby (3) have described three methods for the small-scale generation of chlorine dioxide, based on reacting chlorine gas with solid sodium chlorite. There are some handicaps in the practical use of these procedures. In one method, a 3-hour reaction period for the generation of chlorine dioxide gas is required. In other procedures described for noncontinuous and continuous production of chlorine dioxide, somewhat unreliable results were obtained or undesirable procedures were encountered. For example, for the preparation of chlorine dioxide by the use of chlorine gas and technical flake chlorite, considerable amounts of chlorine had to be passed through the chlorite tower before conditions stabilized. Some chlorine reacted with the soda ash contained in the chlorite and the yields of chlorine dioxide from a given amount of chlorine gas-air mixture passed through the tower could not be reproduced from day to day. Additional experiments showed that the moisture content of the air passed through the tower greatly altered the yield of chlorine dioxide. A continuous method for the generation of chlorine dioxide, described by these authors, required considerable amounts of equipment and numerous calibrations. Also, after the system is started, time is wasted until a constant output is reached. Titrations must be performed to check the rate of production before small samples of flour can be treated with some degree of control.

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[2] Wallace & Tiernan Company, Inc., Belleville, New Jersey.

Two practical methods for the small-scale generation of chlorine dioxide are described in this report.

Diluted Chlorine Dioxide Method

Materials and Methods. For earlier experiments, the Cunningham and Losch method (2), in which pure chlorine dioxide is generated by passing chlorine gas slowly through 25% sodium chlorite[3] solution, was used. Satisfactory results were obtained with 100-ml. glass-stoppered gas washing bottles, preferably two in a series, connected with Tygon tubing, as illustrated in Fig. 1.

The bottles are nearly filled with chlorite solution but space is allowed for bubble formation to avoid any solution spilling over to the next bottle or out of the exit. Chlorine is led from a cylinder equipped

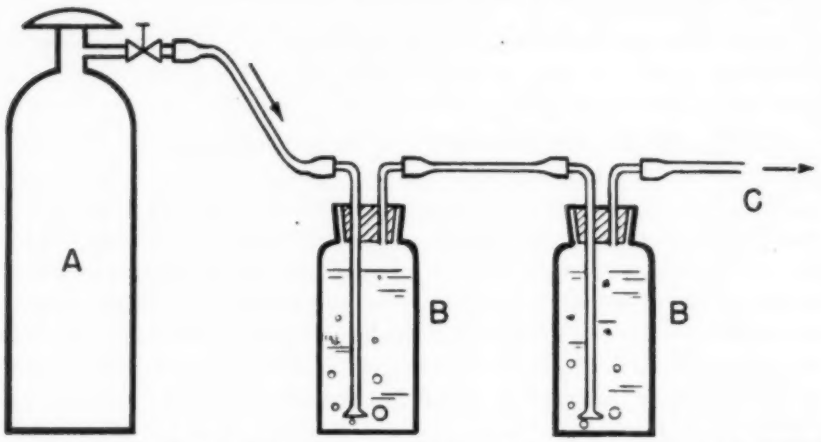

Fig. 1. Schematic diagram of apparatus for production of pure chlorine dioxide gas. A. Chlorine cylinder; B. Gas washing bottles containing 25% sodium chlorite solutions; and C. Pure chlorine dioxide gas exit.

with a needle valve and bubbled through the chlorite solution. Three distinct reaction periods may be observed. At first, an induction period occurs when chlorine is absorbed by the solution and the chlorite solution turns dark. During this early period, the chlorine dioxide coming out of the exit is diluted with air. Second, a stable period is reached when nearly pure chlorine dioxide is delivered from the exit tube. During the third period, the chlorite solution turns from a dark brown to a straw color and chlorine gas begins to evolve.

Samples of pure chlorine dioxide gas are taken from the exit tube during the stable period by filling a pipette or gas burette with the gas. Flour is treated by diluting a calculated amount of pure chlorine

[3] It is important to remember that sodium chlorite solution is very active. Also, the crystalline or flake sodium chlorite should be protected against contamination with organic matter, in order to avoid fires and explosions.

dioxide gas with air. The gas mixture is then passed through a mixing chamber and slowly applied to flour in an Agene Demonstrator Agitator. For varying treatments, a series of pipettes can be calibrated for treating 4 lb. of flour, or given volumes of gas may be taken and the flour weight varied.

For accurate control, samples of the gas should be analyzed by the method of Woodward, Petroe, and Vincent (5), which will be described later in more detail. A useful addition to their method is to buffer the potassium iodide solution to pH 8 with phosphate buffer. Free chlorine and one-fifth equivalent of chlorine dioxide reacts with potassium iodide under neutral and slightly alkaline conditions. Four-fifths equivalent of chlorine dioxide reacts with potassium iodide only under acid conditions. Electronically, this may be illustrated as follows:

$$Cl_2 + 2e \rightarrow 2\ Cl^-$$
$$ClO_2 + e \rightarrow ClO_2^-$$
$$ClO_2^- + 4\ H^+ + 4e \rightarrow Cl^- + 2\ H_2O$$

If the potassium iodide should be slightly acid, some of the four-fifths equivalent of chlorine dioxide may liberate iodine slowly which will titrate as apparent chlorine. Therefore, buffering of the potassium iodide solution is a more accurate procedure. If the titration of iodine liberated at pH 8 is one-fifth of the total titration under acid conditions, pure chlorine dioxide is indicated. If the titration at pH 8 is more than one-fifth of the total titration under acid conditions, the gas is contaminated with chlorine.

Examples of typical titrations of equal volumes of gas taken from the generator and illustrating the induction period, and also the period after stability was reached, are listed in Table I.

TABLE I

ILLUSTRATING INDUCTION PERIOD IN GENERATING CHLORINE DIOXIDE BY PASSING CHLORINE GAS THROUGH SODIUM CHLORITE SOLUTION
(Analyses of successive 5.5 ml. gas samples)

Gas sample	Titer of iodine liberated by ClO_2 with 0.1 *N* sodium thiosulfate	
	pH 8	Acid conditions
No.	*ml.*	*ml.*
1	1.9	9.0
2	2.1	10.1
3	2.1	10.5
4	2.1	10.5

The gas samples were analyzed consecutively at 10-minute intervals.

Table II lists gas analysis values after the generator had reached stable conditions and after excess chlorine had started to evolve.

TABLE II

ANALYSIS OF CHLORINE DIOXIDE GAS GENERATED BY PASSING CHLORINE THROUGH SODIUM CHLORITE SOLUTION

Gas sample No.	Volume of gas sample at 23°C.	Titer of iodine liberated by ClO_2 with 0.1 *N* sodium thiosulfate		Volume of chlorine dioxide found	Volume of chlorine found
		pH 8	Acid conditions		
	ml.	*ml.*	*ml.*	*ml.*	*ml.*
1	25	10.5	50.1	24.0	0.7
2	25	10.3	50.1	24.1	0.5
3	25	10.5	50.4	24.2	0.6
4	25	11.2	48.7	22.3	2.2

Gas sample No. 4 illustrates the break-through period since appreciable amounts of chlorine were found.

Advantages and Disadvantages of This Method. The advantages of this method are the relatively simple setup for the generator and the excellent control obtained when the gas samples are taken after conditions have stabilized.

Among the disadvantages are: (1) the hazards of dealing with nearly pure chlorine dioxide gas; (2) the method is wasteful in respect to the use of chlorite, especially if only one or two samples of flour are treated; (3) the procedure is somewhat cumbersome because it requires an equilibrium condition dependent on time, and needs constant checking of the purity of the gas delivered, if precision is desired.

Caution and Comments. All glassware must be absolutely clean. Rubber stoppers and rubber tubing cannot be used. A hood or other method of disposal of the excess gas must be provided. A partially exhausted chlorite solution should be disposed of *at once* because it is saturated with chlorine dioxide gas which comes out of solution on standing and may then cause explosions.

For mill control work, gas analysis determinations may not be necessary.

Acetic Anhydride Method

A more desirable method for generating chlorine dioxide on a small scale would be to generate the gas in solution and then remove it by aeration. Aston[4] (1) was granted a patent in February, 1948, on the production of chlorine dioxide by reacting organic anhydrides with aqueous solutions of chlorites. A laboratory method for generating chlorine dioxide and for bleaching flour on a small scale has been developed from this reaction. After extensive experimental work, a procedure has been evolved in which the yield of chlorine dioxide is

[4] The use of patent No. 2,436,134 for laboratory purposes is permitted by Mathieson Chemical Corporation through private communication.

proportional to the chlorite used within the range for treating experimental batches of flour. The details of the procedure are as follows:

Reagents:

1. Sodium chlorite solution: 0.1% for 1-lb. treatment and 1% for 4-lb. treatment.
2. Sodium acetate buffer solution, 2 *M:* 164 g. of anhydrous sodium acetate are dissolved in distilled water and made to a volume of 1 liter.
3. Acetic anhydride, 95%.

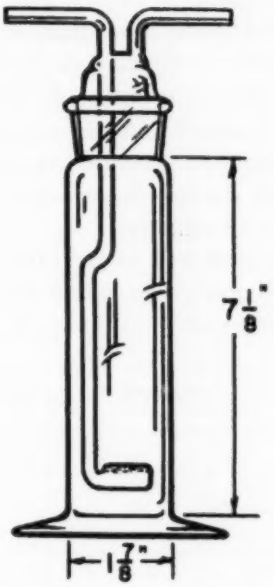

Fig. 2. Chlorine dioxide generator bottle comprising a pyrex gas washing bottle (Inter-joint, capacity 250 ml., stopper 40/35) with fused-in fritted disc (medium or coarse porosity), 20 mm. diameter.

4. Potassium iodide solution, 5%, buffered to pH 8 with phosphate buffer (95 g. disodium hydrogen phosphate and 5 g. potassium dihydrogen phosphate are made to a volume of 1 liter).
   10 ml. of buffer mixture are required for each liter of 5% KI solution. If the pH is not 8 (±0.1) it is adjusted with either the alkaline or acidic constituent of the buffer mixture.
5. Acetic acid solution, 50%.
6. Sodium thiosulfate solution, 0.1 *N*.
7. Starch indicator, 2% solution.

Apparatus:

1. Supply of compressed air or other sources of air, such as foot bellows or hand pressure bulbs.

2. Gas washing bottle with fritted glass diffuser of medium or coarse porosity and 20 mm. diameter; bottle capacity, 250 ml.; ground glass stopper. This bottle is to be used as the gas generator (Fig. 2).
3. Flour agitator such as supplied with the Novadel-Agene Demonstrator unit.

Procedure for the Preparation of a Calibration Curve:

1. The exact concentration of the sodium chlorite solution must be determined by reacting 5 ml. of 1% or 50 ml. of 0.1% aliquots with acidified potassium iodide and titrating the liberated iodine with 0.1 *N* sodium thiosulfate solution.
2. Fifty ml. of acetate buffer are placed in the chlorine dioxide generator, then chlorite solution is added. Finally, 1 ml. of acetic anhydride is added and the generator is closed and shaken vigorously for about 15 seconds. Immediately thereafter, the gas is aerated from the reaction mixture until the waste solution is colorless (about 15 minutes).
3. The gas must be analyzed quantitatively and qualitatively by passing it through two gas washing bottles containing 30 ml. of 5% potassium iodide solution buffered to pH 8. The liberated

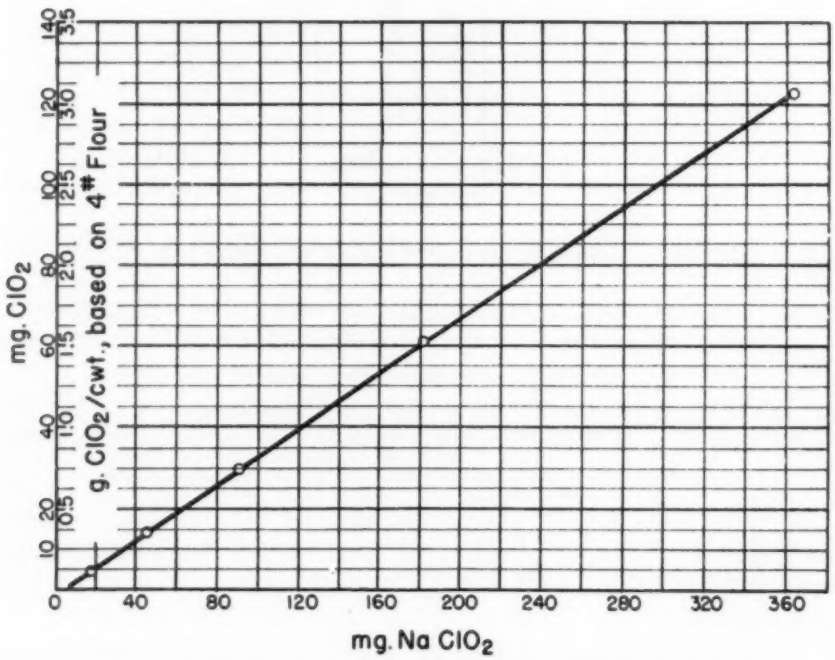

Fig. 3. Calibration curve for treating 4 lb. flour by the acetic anhydride method.

iodine is first titrated at pH 8 with 0.1 *N* sodium thiosulfate in the presence of 1 ml. of starch indicator. A second titration is performed after the same solution has been acidified with 25 ml. of 50% acetic acid solution.

4. Several determinations of chlorine dioxide yields should be made by using different quantities of sodium chlorite solution in order to prepare a calibration curve as shown in Fig. 3.

If the sodium chlorite solution is stored in a brown-colored bottle and out of direct sunlight, it will remain stable for several weeks or longer.

For verification of the sodium chlorite content of solutions: 5 ml. aliquots of 1% chlorite solution, or 50 ml. of 0.1% solution, are mixed with 1 g. of potassium iodide. When the crystals are dissolved, 20 ml. of 50% acetic acid solution are added and the mixture is allowed to stand 5 minutes in a dark place before titration with 0.1 *N* sodium thiosulfate solution.

$$NaClO_2 + 4\ KI + 4\ HC_2H_3O_2 \rightarrow NaCl + 4\ KC_2H_3O_2 + 2\ H_2O + 2\ I_2$$
$$I_2 + 2\ Na_2S_2O_3 \rightarrow 2\ NaI + Na_2S_4O_6$$

Calculations:

Grams of sodium chlorite contained in aliquot = ml. of standard thiosulfate × normality × molecular weight of $NaClO_2/4000$.

For checking the weight of chlorine dioxide produced, the method outlined in point (3) under "Procedure for preparing a calibration curve" is used.

The reaction is presented by the following equations:

$$2\ ClO_2 + 10\ KI + 8\ HC_2H_3O_2 \rightarrow 2\ KCl + 8\ KI(C_2H_3O_2 + 5\ I_2$$
$$I_2 + 2\ Na_2S_2O_3 \rightarrow 2\ NaI + Na_2S_4O_6$$

Calculations:

Grams of chlorine dioxide titrated = 5/4 of acid titration with thiosulfate × normality × molecular weight of $ClO_2/5000$.

In Table III are illustrated the amounts of chlorine dioxide produced with increasing amounts of sodium chlorite when maintaining the foregoing conditions.

To obtain a measure of the reproducibility of the amounts of chlorine dioxide generated, four additional determinations were made with the different amounts of sodium chlorite listed in Table III and the results are summarized in Table IV.

The average values have been employed in the calibration curve shown in Fig. 3 for the treatment of 4 lb. of flour.

The amount of chlorine dioxide treatment per 100 lb. of flour when 4 lb. of flour are used in the experimental agitator can be read on the vertical axis.

TABLE III

GENERATION OF CHLORINE DIOXIDE FROM SODIUM CHLORITE BY REACTION WITH ACETIC ANHYDRIDE; CHLORINE DIOXIDE PRODUCED FROM VARYING AMOUNTS OF SODIUM CHLORITE

$NaClO_2$	Titer of iodine liberated by ClO_2 with 0.1 *N* sodium thiosulfate		ClO_2 generated
	pH 8	Acid conditions	
mg.	*ml.*	*ml.*	*mg.*
18.1	1.0	3.8	4.7
45.3	2.6	11.4	14.9
90.6	4.8	22.9	30.6
181.2	9.9	47.2	62.2
362.4	20.9	97.5	129.3

Numerous cereal laboratories mill 1000-g. samples of wheat experimentally and about 500 to 700 g. of flour are obtained from each test. To treat such small quantities of flour, the calibration curve shown in Fig. 4 has been prepared for use with 1-lb. samples of flour. This curve is based on the data given in Table V. The conditions of generation were the same as previously described, except a 0.1% sodium chlorite solution was used.

Application of Gas to Flour. The component parts of the apparatus for treating flour by the acetic anhydride method are schematically represented in Fig. 5. If a source of compressed air is at hand, a needle valve is used for regulating the air flow through the system, but pressure bulbs with a reservoir or foot bellows can also be used. It is advisable to use a screw clamp in the air-supply line for regulating the air flow.

The chlorine dioxide needed to treat a 4-lb. sample or a 1-lb. sample of flour and the amounts of sodium chlorite needed for generating such quantities of gas may be read from the calibration curve. When treating flour, the chlorine dioxide gas diluted with air is passed into

TABLE IV

GENERATION OF CHLORINE DIOXIDE FROM SODIUM CHLORITE BY REACTION WITH ACETIC ANHYDRIDE

(Results of experimental trials made on five different days)

$NaClO_2$	ClO_2 generated					Mean
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
18.1	4.7	5.0	4.6	4.5	5.0	4.8
45.4	14.9	14.1	14.7	14.0	14.1	14.4
91.0	30.6	29.6	30.5	30.2	30.4	30.3
182.0	62.2	62.2	62.8	62.9	62.2	62.5
364.0	129.3	126.9	124.1	124.1	126.0	126.1

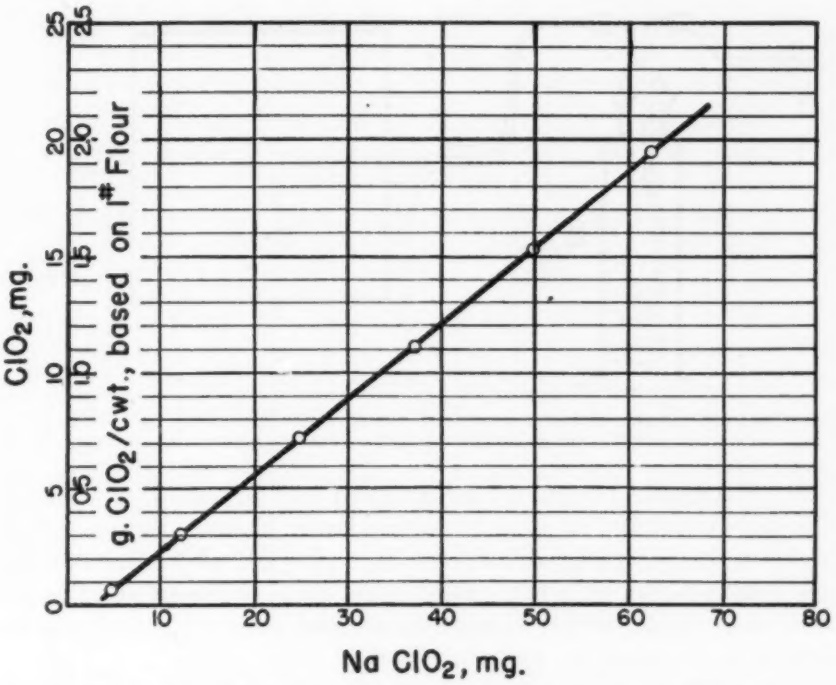

Fig. 4. Calibration curve for treating 1 lb. flour by the acetic anhydride method.

the flour agitator and aeration continued until the reaction mixture becomes colorless (10 to 15 minutes).

An air current of approximately 1 liter per minute is used for stripping the gas from solution. Less air will require a longer time, whereas more air will shorten the aeration time and there is danger of forcing chlorine dioxide out of the agitator before it has reacted with the flour.

Comments on Operation. The solution after aeration has a pH of 5.5 to 6.0 and has an iodine equivalent of 0.3 ml. 0.1 *N* sodium thiosul-

TABLE V

GENERATION OF CHLORINE DIOXIDE FROM SODIUM CHLORITE BY REACTION WITH ACETIC ANHYDRIDE

(Data for treating 1-lb. samples of flour with chlorine dioxide)

$NaClO_2$	ClO_2 generated					Mean
mg.	mg.	mg.	mg.	mg.	mg.	mg.
5.0	0.7	0.8	0.8	0.7	0.7	0.7
12.4	3.1	3.1	3.2	3.0	3.1	3.1
24.9	7.1	7.2	7.3	7.3	7.1	7.2
37.2	11.0	11.1	11.0	11.0	11.5	11.1
49.8	15.2	15.5	15.2	14.8	15.6	15.3
62.3	19.6	19.5	19.7	19.3	19.6	19.5

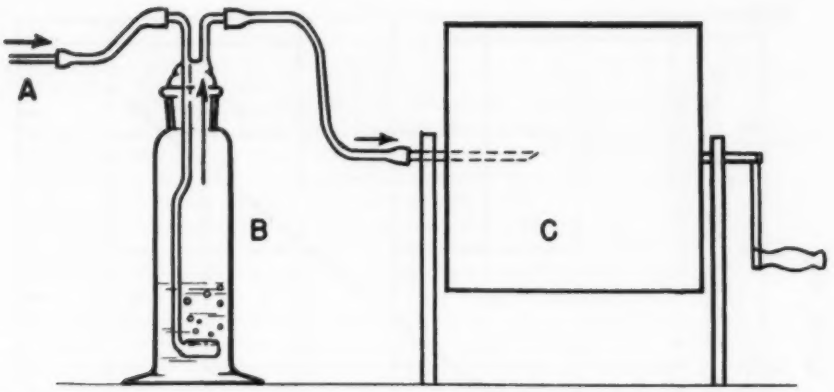

Fig. 5. Schematic diagram of apparatus for the laboratory treatment of flour by the acetic anhydride method. A. Air inlet (1 liter per min.); B. Generator bottle; and C. Flour agitator.

fate. A number of variables influenced the liberation of chlorine dioxide. When 100 ml. of 2 *M* acetate buffer were used instead of 50 ml. there was no appreciable alteration in the yield of chlorine dioxide. If less than 50 ml. of buffer solution were used, the waste solution became more acid, the yields were lower, and a longer period was required for stripping the gas from solution.

The addition of saturated sodium chloride solution to the reaction mixture increased the chlorine dioxide yield and decreased the aeration time somewhat; however, the resultant gas contained more apparent chlorine.

The use of less than the specified amount of acetic anhydride reduced the yield, but larger amounts served no useful purpose.

Glass or Tygon tubing should be used for conducting the gas from the generator to the agitator. Rubber stoppers, rubber tubing, or diffusers made of plastics are not recommended. Before using any new equipment it is important to saturate material which reacts with chlorine dioxide, in order that the flour be accurately treated.

The concentration of chlorine dioxide gas in the air emerging from the generating bottle was found to be very low, as shown by the

TABLE VI

CONCENTRATION OF CHLORINE DIOXIDE IN AIR WHILE TREATING 4-LB. FLOUR WITH 2 G. CHLORINE DIOXIDE PER CWT.

Time at which gas samples were taken	Chlorine dioxide (by volume) %
At once	1.2
After 5 minutes	0.8
After 10 minutes	0.1

typical analysis recorded in Table VI. This percentage of chlorine dioxide-in-air is far below the level where any explosion could possibly occur and compares favorably with commercial generation in the mill where 0.5% by volume is used.

Advantages and Disadvantages. The acetic anhydride method of generating chlorine dioxide was found to be safe and reliable. After a calibration curve has been prepared, small samples of flour can be treated in a very short time at any desired practical level. The reagents used for this purpose are quite stable and only an occasional titration is necessary for measuring the sodium chlorite content of the stock solution. There is no hazard due to the use and presence of compressed gases in the laboratory.

The chief disadvantage appears to be the use of an all-glass gas washing bottle with a fragile glass tubing holding the diffuser. Also, the comparatively long aeration period is a disadvantage in laboratories where a supply of compressed air is not available and when the flour agitator is turned by hand.

Comparison of Treatments with Pure Chlorine Dioxide and Gas Generated by the Acetic Anhydride Method

To show that both methods produced the same results, flours were treated at equal levels with chlorine dioxide. Color removal was measured by the Pekar test and by the extraction and determination of carotene, using water-saturated normal butyl alcohol.

TABLE VII

COMPARISON OF BLEACHING RESULTS OBTAINED WITH VARIOUS DOSAGES OF CHLORINE DIOXIDE GENERATED BY THE DILUTED CHLORINE DIOXIDE AND ACETIC ANHYDRIDE METHODS

Flour	Treatment ClO_2/cwt.	Carotene in flour	
		Dilution method	Acetic anhydride method
	g.	*ppm.*	*ppm.*
Patent	0.64	0.95	0.97
Clear, highly pigmented	2.86	1.25	1.23
Patent	0.25	1.57	1.52
Patent	0.69	0.98	0.94

The results recorded in Table VII show that there is no significant difference between the two methods. The dosages which were employed are not necessarily optimum for the best maturing action.

Further studies of the methods and also the relation between laboratory and mill treatments are being continued.

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THE DETERMINATION OF PERSULFATE IN FLOUR AND DOUGH[1]

M. E. AUERBACH, H. WM. ECKERT, and ELEANOR ANGELL[2]

ABSTRACT

A method has been developed for the quantitative determination of as little as 5 to 10 parts ammonium persulfate per million parts of flour or dough. The method involves the re-oxidation of leuco-fluorescein to fluorescein through the action of persulfate ion. Under controlled conditions, the intensity of the developed fluorescence bears a reproducible relationship to the persulfate content of the sample.

As part of an investigation designed to assess the utility of ammonium persulfate [$(NH_4)_2S_2O_8$] as a flour maturing agent, it seemed advisable to determine the stability of small amounts of persulfate in flour and dough. It was postulated that amounts of the order of 50 to 200 parts per million might be necessary to mature flour. It follows that an analytical method, to yield useful results, should have quantitative value even at a level as low as 10 parts per million.

The literature reveals no dearth of qualitative color tests for persulfate ion. Aside from the well known benzidine test, various workers have reported the use of fuchsin, brucine, strychnine, iodides, diamino-diphenyl amine, diaminofluorene, aniline sulfate, methylene blue, indigo and ortho-tolidine. Diaminodiphenylamine and diaminofluorene were not available to us. Of the other reagents, only o-tolidine (1) showed up well enough in exploratory tests to merit careful study. This reagent has two important advantages over the commonly used benzidine: not only is it considerably more sensitive, but the blue oxidation product is soluble in water. As little as one microgram of ammonium persulfate in one gram of water oxidizes o-tolidine to a definite blue coloration. However, in one gram of flour, we were unable to detect amounts of persulfate equivalent to less than

[1] Presented at Annual Meeting, May 1949; received for publication May 19, 1949.
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35 or 40 parts per million, and were thus reluctantly forced to abandon this reagent as insufficiently sensitive for our purpose. A number of other potentially useful reagents, not specifically mentioned in the literature, were also investigated. Among these were the leuco (i.e., reduced) forms of malachite green and crystal violet. Neither of these was found to be as sensitive as o-tolidine, but they are mentioned because they suggested the compound eventually chosen.

If colorimetric methods are put aside as being insufficiently sensitive, it becomes natural to search for a fluorometric method. The reason for this is that modern instruments for the quantitative measurement of fluorescence intensities are relatively more sensitive than corresponding instruments for measuring color intensities. Having in mind the behaviour of leuco-crystal violet, it seemed possible that the reduced form of some fluorescent dye might fulfill our requirements. Perhaps the commonest of this class of dyes—the sodium salt of fluorescein—turned out to be eminently satisfactory. Reduced fluorescein is not fluorescent, but is easily re-oxidized to the parent compound which is, of course, very intensely fluorescent.[3]

Using reduced fluorescein as test reagent, it was found possible to detect as little as one part ammonium persulfate per million of flour. In order to set up reproducible quantitative assay conditions, it was necessary first to investigate methods for separating or extracting minute amounts of persulfate from large amounts of interfering organic substance—i.e., flour. Previous experience with biochemical assay methods suggested the method which will now be described in detail.

Required Reagents

1. Sodium fluorescein. Dissolve 0.2 g. in 100 ml. of water.
2. Sodium potassium tartrate. Dissolve 10 g. of the U.S.P. grade in 100 ml. water.
3. Titanous chloride volumetric solution, 0.04 Normal.

General directions for preparation and storage are given in several texts, of which perhaps only one (3) need be mentioned. The solution must be kept under hydrogen. Analytical grade titanous chloride (20%) distributed by Fisher Scientific Co. has proved very satisfactory as a stock reagent.

4. Zinc sulfate solution, 0.3 Normal.
5. Sodium hydroxide solution, 0.1 Normal.
6. Filtercel (filtering aid).
7. Ammonium persulfate standard solution, 5 micrograms per ml. Dissolve exactly 100 mg. C.P. anhydrous ammonium persulfate in

[3] When the work to be described had been practically completed (December, 1948) our attention was drawn to a Russian paper (2) describing leuco-fluorescein as a reagent for oxidizing agents.

exactly 200 ml. distilled water. Dilute 5 ml. of this solution to exactly 500 ml., using distilled water. At the final dilution the reagent does not keep well. It is recommended that it be used within 3 hours of preparation.

8. Reduced fluorescein: given below.

Procedure

I. Setting Up the Standard Curve

To each of five 125 ml. Erlenmeyer flasks add 1.0 g. of untreated (persulfate-free) flour of the type to be tested. Add to each flask 0.5 g. of filtercel and mix. Add, respectively, 34, 33, 32, 30, and 26 ml. of water. Add, respectively, 0, 1, 2, 4, and 8 ml. of the standard ammonium persulfate solution. Add to each flask 4 ml. of the zinc sulfate solution, swirl, and then add 12 ml. of the sodium hydroxide solution. (At this point, the mixture should be very faintly acid. If it is alkaline, the subsequent filtrate will be turbid.) Stopper each flask and shake occasionally during an interval of 15 minutes. Filter each through Reeve Angel No. 230 or equivalent paper. Transfer 10 ml. of clear filtrate from each flask to a fluorimeter tube. Add to each tube exactly 0.2 ml. of reduced fluorescein reagent,[4] prepared as follows:

Add to a 15 ml. centrifuge tube, in order, 1 ml. stock sodium fluorescein solution, 5 ml. of the sodium potassium tartrate solution, and 4 ml. of the titanous chloride solution. Cap the tube, mix the contents well, and spin in the centrifuge until supernatent is clear (10 minutes at 2,000 r.p.m. seems sufficient).

Mix the contents of each fluorimeter tube, and allow to stand at room temperature for 30 minutes. Set the instrument to zero with the blank flour extract, then read in turn the fluorescence intensity of each extract. The authors use the Coleman Photofluorometer Model 12A with primary filter B-1-S, secondary PC-9. From the readings construct a curve. Figure 1 represents typical curves.

These readings represent the oxidizing effect of extracts of flour treated at levels of 5, 10, 20, and 40 parts ammonium persulfate per million of flour, respectively. The aliquots of filtrate actually used contain only 1, 2, 4, and 8 micrograms ammonium persulfate, respectively. Amounts larger than these produce fluorescence too intense to be measured conveniently.

The flour represented by curve A was vitamin enriched, containing (by actual analysis) in each pound, 2.18 mg. thiamine chloride, 1.36 mg. riboflavin and 17.3 mg. niacin.

[4] This reagent should be prepared just prior to use. It can just comfortably be made ready during the 15 minute waiting period.

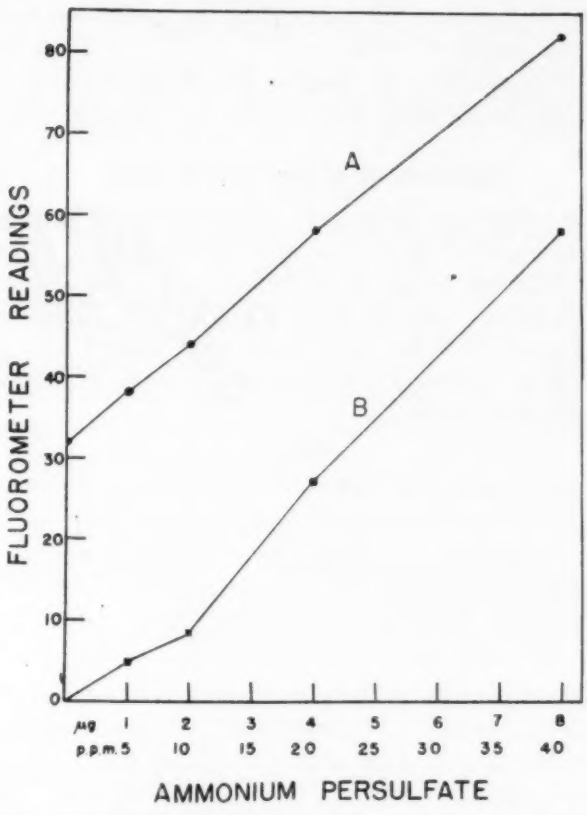

Fig. 1. Determination of ammonium persulfate in flour: fluorometer readings of extracts from standard 1 gram samples. A. Vitamin enriched flour. B. Non-vitamin enriched flour.

Curve B represents a non-enriched flour. The relationship between the two curves will be brought out in the summary discussion.

The intrinsic fluorescence developed by given standard amounts of persulfate varies slightly from day to day, and so it is necessary to run a standard series every day, and preferably, with each series of test samples.

II. Assay of Samples

Along with the standard series already described, process 1-gram samples of the treated flours to be tested, without, of course, adding standard persulfate. If it is known, or suspected, that the treated flours contain more than 40 parts ammonium persulfate per million of flour, then, instead of taking a 10 ml. aliquot of the zinc hydroxide filtrate, take a correspondingly smaller aliquot. In this case, however, make up the difference (i.e., 10 ml. minus aliquot actually taken) with filtrate from the flour blank used in the standard series. This is

important, to compensate for quenching and reduction errors. Plot results from the standard curve.

Portions of dough may be assayed in exactly the same way, with special attention being given to breaking up lumps at the beginning of the test.

Applications of the Test Method

Using the technique described, the stability of ammonium persulfate in non-enriched flour has been determined. Two mixtures, representing different levels of maturing agent, were obtained from the Doty Technical Laboratories, Kansas City, Missouri. These samples, contained in 5 lb. cloth bags, were stored in a laboratory cupboard, with no special precautions, and assayed at intervals. The results are shown in Table I.

TABLE I

THE STABILITY OF AMMONIUM PERSULFATE IN FLOUR

	Sample W-105	Sample W-106
Labeled content, September 24, 1948	66 p.p.m.	132 p.p.m.
Assay of November 3, 1948	70 p.p.m.	135 p.p.m.
Assay of January 24, 1949	70 p.p.m.	140 p.p.m.
Assay of April 12, 1949	68 p.p.m.	145 p.p.m.

The addition of water to flour was found to have a profound effect on the persulfate content. A dough was prepared by adding 60 parts of water to 100 parts of flour which contained 200 parts ammonium persulfate per million. Portions of this mixture were incubated in small closed containers (to conserve water vapor) for various lengths of time at about 27°C. (80°F.) and 37°C. (100°F.) respectively, and then assayed for ammonium persulfate. Table II summarizes the results.

TABLE II

THE STABILITY OF AMMONIUM PERSULFATE IN MOIST FLOUR

Time in minutes	27°C.	37°C.
0	200 p.p.m.	200 p.p.m.
8	55 p.p.m.	17 p.p.m.
15	27 p.p.m.	5 p.p.m.
30	24 p.p.m.	0
45	15 p.p.m.	0
60	0	0

In another test, a dough was prepared by mixing by weight 60 parts water, 1 part sodium propionate, 6 parts dextrose, 2 parts shortening, 2 parts salt, 2 parts yeast, and 100 parts flour which contained 1,000 parts ammonium persulfate per million.[5] The

[5] This grossly overdosed flour was used deliberately, to see how much persulfate might survive the test conditions.

sample was incubated at about 27°C. (80°F.) for three hours, at the end of which time an assay showed the residual persulfate to be 7 parts per million. The same sample was then incubated for an additional 30 minutes at about 60°C. (150°F.). At the end of this time an assay showed no persulfate to be present.

Discussion

A method for the assay of ammonium persulfate in flour has been described. The results of a preliminary study indicate that ammonium persulfate is virtually completely stable in normally dry flour, but that it decomposes rapidly in the presence of added water.

A large number of successful assays have convinced the authors that the method has reasonably good precision. Thus, flours carefully prepared so as to contain 100 parts per million ammonium persulfate have been found to assay 100 ± 10 p.p.m. Flours containing only 10 parts per million assay 10 ± 2.5 p.p.m. Readings indicating levels below 5 p.p.m. have little meaning, especially if a control flour of the same type as the unknown is not available. It should be noted that relatively few distinctly different samples of flour were involved in this investigation. It is entirely possible that some types of flour may require modification of the general method. For example, it was noticed that with a very few samples, more than the recommended amount of zinc hydroxide was required to bring about a clear zinc filtrate.

Vitamin enrichment constitutes an obvious source of complication in the method. But, as indicated in curves A and B, Fig. 1, it is easy to make the necessary adjustment. Curve A, representing additions of persulfate to an enriched flour,[6] is substantially parallel to curve B, representative of a non-enriched flour. Clearly, the presence of vitamin enrichment involves nothing more than a simple additive effect. The "apparent persulfate" value of the normal enrichment concentration (represented by the first point on curve A) amounts to about 23.5 parts per million of flour. Obviously, the simplest way to cancel out the vitamin effect is to use an enriched, non-persulfated flour as the blank when assaying persulfate-matured enriched flours. Even if the enrichment level of this reference sample lies as much as 20% above or below that of the test flour, the net error from this source will probably be not more than ± 5 p.p.m. as persulfate—i.e., 20% of 23.5 p.p.m.

The chemistry involved in the analytical technique is, very briefly, as follows: in the presence of tartrate, fluorescein ($C_{20}H_{12}O_5$) is reduced

[6] This flour was assayed and found to contain, per pound, 2.18 mg. thiamine chloride, 1.36 mg. riboflavin, and 17.3 mg. niacin.

quantitatively (4) by titanous ion to fluores*cin* ($C_{20}H_{14}O_5$). The tartrate probably functions both as a buffer and as a reduction catalyst. The fluores*cin*, which is not fluorescent, is then allowed to react with persulfate ion, and is thereby oxidized back to vividly fluorescing fluorescein. One point, however, is not readily apparent, and should be made clear. A relatively large excess of titanous chloride is used to reduce fluorescein in preparing the reagent. Clearly, if any titanous ion, as such, existed in the reagent at the moment it was added to the flour persulfate extract, a certain amount of the persulfate would react with the trivalent titanium instead of the reduced fluorescein, and thus an apparently low assay would result. As a matter of fact, no titanous ion whatever is left in the reagent after it has been mixed and centrifuged. Presumably, the excess is used up by air in the centrifuge tube. The absence of titanous ion in the prepared reagent was demonstrated by adding 2 ml. of the mixed and centrifuged reagent to 100 ml. of distilled water. The solution was mixed by bubbling purified carbon dioxide (such as used for titanous chloride titrations) through the container for a few seconds. On the addition of one drop of 0.01 *N* methylene blue, the solution became permanently colored.

The effect on the proposed method of concomitant benzoyl peroxide or potassium bromate was studied briefly. Amounts of benzoyl peroxide up to 150 p.p.m. have a negligable effect, if any, on the persulfate assay. Bromate, however, definitely interferes. Three to four p.p.m. of potassium bromate yield an apparent ammonium persulfate content of about one p.p.m.

Acknowledgments

The authors wish to acknowledge their debt to Mr. J. M. Doty for his kindness in preparing and supplying several test mixtures of flour, and to Dr. C. M. Suter of these laboratories for his valuable suggestions, which led to the use of reduced fluorescein as a test reagent.

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A NOTE ON THE OCCURRENCE AND ELIMINATION OF FORMALDEHYDE FUMES FROM A BAKE LABORATORY ATMOSPHERE [1]

D. K. CUNNINGHAM and I. HLYNKA

ABSTRACT

The principal irritant in noxious fumes produced during baking under certain conditions has been identified as formaldehyde formed from the alcohol, vaporized from the loaves during baking, by the catalytic action of open heating elements of nichrome wire in the oven.

Redesigning of our baking laboratory recently involved moving the oven to an ill-ventilated position. Thereafter, noxious fumes irritating to the eyes and respiratory tract became noticeable during baking. A preliminary search of the literature yielded no information on this topic, and an experimental investigation was therefore undertaken.

Preliminary observations on the odor and physiological effect of the fumes indicated that the irritating substance might be formaldehyde. To test this hypothesis the unknown material was concentrated by scrubbing the laboratory air during baking. A suitable scrubbing column consisted of an air filter to remove dust, followed by a sintered glass disc through which air was drawn by suction into a column (20 cm.) of 100 ml. freshly boiled distilled water; unboiled distilled water gave a slight reaction for aldehydes with sensitive reagents.

The unknown solution was tested with Tollens ammoniacal silver reagent (3) and Schryver's phenyl hydrazine reaction (2). The Tollens' silver reagent was prepared according to an improved method by mixing equal volumes of 10% silver nitrate and sodium hydroxide solutions and then adding ammonium hydroxide dropwise until the brown precipitate just disappeared. When this reagent was added in equal volume to the unknown solution, a brownish color formed and a a precipitate of reduced silver was deposited on standing. The test was exceedingly sensitive but required several hours for completion.

The Schryver reagent proved much more adaptable. The original method was modified as follows to improve the sensitivity and reproducibility: To 10 ml. of test liquid were added 2 ml. of 1% phenylhydrazine hydrochloride. This mixture was heated in a boiling water bath for three minutes and cooled to room temperature. One ml. of

[1] Manuscript received August 12, 1949.
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5% potassium ferricyanide was then added. After exactly 30 seconds, 5 ml. of chilled hydrochloric acid were run in and the solution was cooled at once in an ice-bath. A red color formed, which was stable for several days in the cold. This reaction gave a positive indication with as little as 0.02 μgm. of formaldehyde, and the sensitivity could have been increased by extracting the red substance with water-saturated normal butyl alcohol.

To determine its specificity, the Schryver test was applied to furfural, acetaldehyde, formic acid, and water saturated with carbon dioxide. Furfural gave a brown color, as did acetaldehyde, although the latter faded rapidly; no color reaction was obtained with formic acid. Since large amounts of carbon dioxide are given off in the baking of bread, its effect was tested by passing carbon dioxide generated from dry ice through the apparatus for six hours. No reaction with Schryver's reagent could subsequently be observed in the solution.

The red color developed by both the test solution and formaldehyde on treatment with Schryver's reagent was examined spectrophotometrically. The concentration of formaldehyde used was one μgm. per ml., which approximated the concentration of unknown in the absorption fluid. Readings were made in a Beckman spectrophotometer at the narrowest slit-width, using 1 cm. corex cells.

Fig. 1 shows the curves obtained by plotting per cent transmission against wavelength over the range 380 to 600 mμ. They are practically identical; the slight variations are due to the difference in concentration of the known and the unknown. The point of minimum transmission for both substances is 523 mμ and is most clearly defined. A maximum occurs at about 400 mμ and is attributed to phenylhydrazine-ferricyanide complex.

The demonstrated specificity of Schryver's reagent (1) together with the similarity of the absorption spectra may be regarded as good evidence that the principal irritant in the bake laboratory atmosphere was formaldehyde.

Several hypotheses concerning the source of the formaldehyde were entertained, but it was finally shown that formaldehyde was formed from the alcohol vapors volatilized from yeasted dough during baking. The open heating elements of nichrome resistance wire served as efficient catalysts. Experiments establishing this information are outlined below.

A muffle furnace, lined with an alundum shell and having no open elements or metal parts likely to possess catalytic activity, was set in a closed fume hood to confine the vapors given off during baking. A set of 18 pup loaves was baked in batches of three. Organoleptic judgment by several individuals revealed only the odor of dough and of

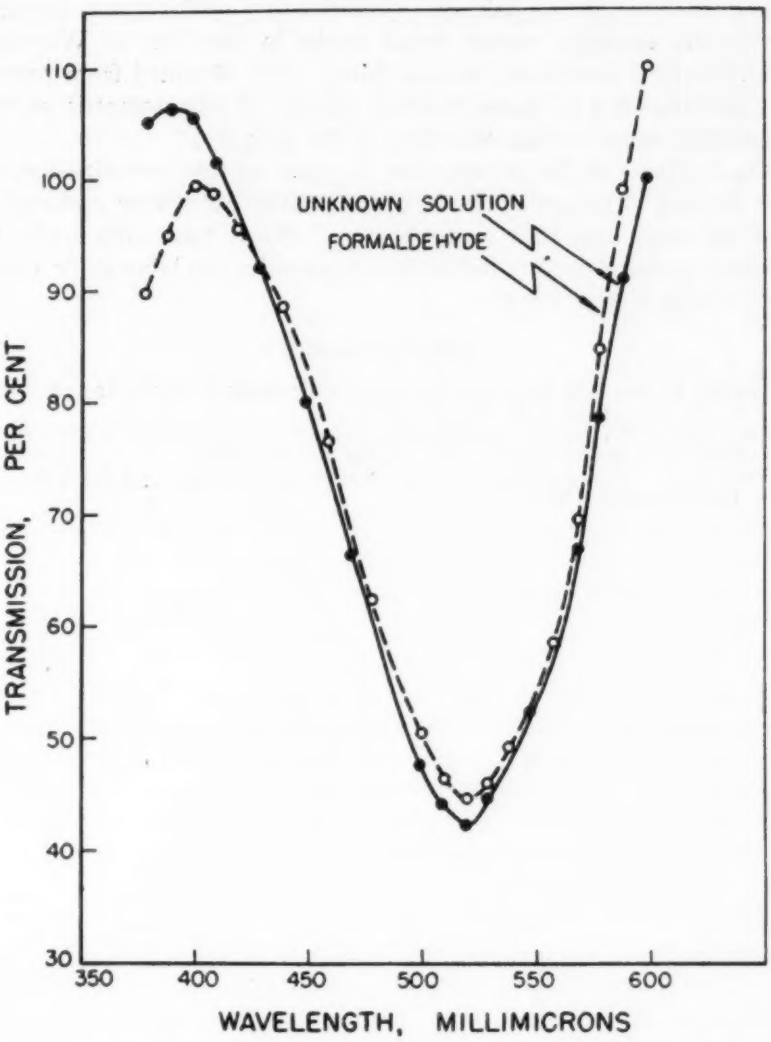

Fig. 1. Comparison of the color complex of formaldehyde and unknown with Schryver's reagent.

freshly baked bread; no irritant was produced. The metal parts of our bake oven were thus definitely implicated.

It was then demonstrated that characteristic acrid fumes were produced by injecting alcohol vapor into the bake oven. A set of 25 doughs, each of approximately 160 g., was prepared by mixing flour with 5% aqueous ethanol. These doughs were panned and fed into the oven, one every five minutes, as is the usual practice. Before long the familiar acrid fumes pervaded the bake laboratory. Ethyl alcohol normally produced in yeast fermentation, was thus the obvious source

of formaldehyde during baking. Confirmatory evidence was obtained incidentally during a recent bread strike in the City of Winnipeg. Several reports describing noxious fumes were obtained from persons who had resorted to home baking. Many of the domestic electric ranges have open heating elements in the oven.

On the basis of the information obtained in this investigation, the open heating elements in the laboratory bake oven were replaced by anodized aluminum-clad strap heaters. Since that time only the pleasant aroma of freshly baked bread pervades the laboratory atmosphere during baking days.

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EFFECT ON DOGS OF FEEDING FLOUR TREATED WITH CHLORINE DIOXIDE AND NITROGEN TRICHLORIDE [1]

FRANK I. NAKAMURA and MARK L. MORRIS [2]

ABSTRACT

Flour treated with chlorine dioxide ranging from 0.61 to 50 g. per hundredweight was fed as a major ingredient in the diet of young and mature dogs for periods ranging from 147 to 355 days.

Young dogs made satisfactory gains in bodyweight while mature dogs were maintained in good physical condition during the tests. Symptoms of canine hysteria were not observed in any of the 12 dogs fed flour treated with chlorine dioxide. All animals remained apparently normal during the tests and showed no clinical abnormalities.

Feeding of flour treated with nitrogen trichloride induced canine hysteria; the time required and the severity of symptoms depended upon the nitrogen trichloride treatment.

It may be summarized that under the condition of experimentation the feeding of flour treated with chlorine dioxide does not induce canine hysteria in either growing or mature dogs.

An affliction, designated by various investigators as canine hysteria, fright fits, and running fits, has been observed under different conditions in dogs during the past 25 years. The exact cause, however, had not been ascertained until Mellanby (3) reported that the feeding of flour treated with nitrogen trichloride (agene), heretofore used widely as an improving agent, developed symptoms of canine hysteria in dogs. The original findings of Mellanby (3) were subsequently confirmed by Mellanby (4), Moran (5), Newell *et al.* (6, 7), Radomski *et al.* (10), and Silver *et al.* (11). The investigation revealed further that rabbits, cats, monkeys, and ferrets are also susceptible to nitrogen trichloride toxicity, while guinea pigs, rats, and chicks were found nonsusceptible. No untoward effect has been found when excessive amounts of highly over-treated flour were fed to humans (2, 6, 9).

The fear that the continuous consumption by man of flour treated with nitrogen trichloride may endanger people's health led to exploration for suitable chemical agents free from toxic effects. Among chemical agents studied chlorine dioxide appeared to be the most suitable agent to replace nitrogen trichloride. Newell *et al.* (6) fed flour treated with 2 g. chlorine dioxide per hundredweight to 3 young

[1] Manuscript received August 3, 1949.

[2] Raritan Laboratories, Inc., Metuchen, New Jersey.

The authors express their appreciation to the staff members of the laboratory who have helped faithfully to complete the work reported in this paper.

dogs for a period of 84 days without any evidence of toxicity. Radomski *et al.* (10) fed flour treated with 0.6 and 1.8 g. chlorine dioxide per hundredweight to two groups of 3 dogs for a comparatively short period of less than 3 weeks without apparent toxicity. The nontoxicity of flour treated with chlorine dioxide was also observed by Arnold (1) and by Newell *et al.* (8). Arnold fed flour treated with 5 g. chlorine dioxide per hundredweight to 5 young dogs for a period of 16 to 28 days. Newell *et al.*, however, fed flour treated with 0.5 to 4 g. chlorine dioxide per hundredweight to 8 young dogs for a period of 12 weeks. Rabbits, monkeys and rats grew well and appeared normal throughout the tests. Thirteen healthy male and female subjects were fed daily, in addition to a normal diet, 55 g. of wheat proteins treated with chlorine dioxide for a period of 6 months without exhibiting abnormal symptoms.

The object [3] of this paper is to present a summary of studies on the feeding of flour treated with chlorine dioxide and nitrogen trichloride. The feeding tests, using dogs, were continued longer than any previously reported studies to determine the effect of the continuous feeding of chlorine dioxide treated flour.

Experimental Method

Young growing and mature dogs were used. The young dogs were immunized against canine distemper during the tests. The mature dogs were previously immunized against distemper. All dogs were clinically normal at the start of the tests. Two dogs were used in each feeding trial.

Stools were checked for parasites once a month, and if positive, rechecked in 10 to 14 days after treatment had been administered. Young dogs were weighed and their temperatures taken twice weekly. Weighing and temperature readings for the mature dogs were done once a week. All dogs were checked daily, in the morning, for general condition such as eye expression, body movement and reflexes, to determine the presence of symptoms of canine hysteria. Samples of blood were examined once a month for hemoglobin concentration, red cell count, white cell count, and Schilling differential counts.

The term "canine hysteria" was used in examination of the test animals to indicate symptoms produced in dogs by feeding flour treated with nitrogen trichloride. Symptoms produced are first general lethargy and muscular incoordination, and later violent running and

[3] This investigation was supported entirely by Wallace and Tiernan Products, Inc., Newark, New Jersey. The material reported in this publication was taken from the data submitted by Wallace and Tiernan Products at a public hearing held in Washington, D. C. by the Food and Drug Administration in October, 1948. The entire program was carried out in cooperation with Dr. L. Reiner of Wallace and Tiernan Products. The authors wish to express their appreciation to Wallace and Tiernan Products for permission to publish the material reported.

barking fits, and in severe cases clonic convulsions followed by death. In some instances animals in severe condition recover completely as far as general appearance is concerned.

The basal ration had the following ingredients in parts: untreated flour 70, lactalbumin 6, sodium caseinate 6, U.S.P. No. 1 salt mixture 4, dextrose 5, lard 8, sodium carboxymethylcellulose 1, and fish liver oil 0.5 (2000 units A and 400 units D per g.). Each 1000 g. of the basal ration contained the following vitamin supplements: thiamin hydrochloride 21 mg., riboflavin 16 mg., nicotinic acid 105 mg., calcium pantothenate 142 mg., pyridoxine hydrochloride 26 mg., choline chloride 1310 mg., and a trace of Menadione.

The experimental ration was prepared by replacing the untreated flour with the test flour. All rations were prepared at an interval of 10 to 14 days. The dry ration was mixed with boiling water in definite proportions just prior to feeding. Boiling water was added to dextrinize the starch in the wheat flour and to increase palatability. In order to insure an adequate intake of vitamin A, 0.5 cc. fish oil was added to the daily food twice a week. Young growing dogs up to 6 months old were fed two or three times a day. All other dogs were fed once a day. The amount of food fed to each animal was determined by the appetite of the animal. The uneaten portion of food was weighed at the end of the day and the weight of the dry ration was calculated and recorded. In most instances, in order to accustom dogs to the type of rations fed, the basal ration was fed prior to the test. All dogs, however, received a colony ration while not on test. It was formulated and prepared in the laboratory to replace commercial dog food usually fed as a colony ration.

All flours were shipped to the laboratory under code number and their identity was not revealed until all tests were completed.

The description of dogs used and flours fed are summarized in Table I. Flour was treated with 0.61, 0.72, 1.83, and 50 g. chlorine dioxide per hundredweight. Gluten was prepared from flours treated with 0.61 and 1.83 g. chlorine dioxide per hundredweight and was incorporated in the ration at a 15% level, replacing the treated flour. Three rations were prepared with flours treated with 3, 20 and 30 g. nitrogen trichloride per hundredweight.

Results and Discussion

The results of the feeding of rations containing 70% flour treated with either chlorine dioxide or nitrogen trichloride are summarized in Table I. The feeding trials lasted from 147 to 355 days. All animals accepted rations containing chlorine dioxide treated flour. Young

TABLE I

DESCRIPTION OF DOGS AND FLOUR FED
SUMMARY OF FEEDING TRIALS

Dog no.	Description of dog species, sex, age and weight at start of test			Flour in diet	Treatment	No. of days on test	Average daily food offered	Average daily food consumed	Weight change during test	Canine hysteria
				%	*per cwt.*		g.	g.	kg.	
663	B F [1]	15 mo.	6.00 kg.	70	Untreated	122	209	200	+1.7	None
470	F F [1]	22 mo.	4.50 kg.	70	Untreated	123	199	193	+1.0	None
582	B F	7 mo.	6.5 kg.	70	0.61 g. ClO_2	189	219	214	+1.0	None
596	F F	5.5 mo.	6.25 kg.	70	0.61 g. ClO_2	189	207	205	+2.1	None
605	F M	3 mo.	3.5 kg.	55	0.61 g. ClO_2					
				15	Gluten from same flour	169	174	161	+2.32	None
520	F F	18 mo.	5.5 kg.	55	0.61 g. ClO_2					
				15	Gluten from same flour	123	229	226	+0.65	None
564	F F	10.5 mo.	4.9 kg.	70	1.83 g. ClO_2	189	190	184	+0.25	None
594	F F	5 mo.	5.0 kg.	70	1.83 g. ClO_2	147	186	170	+1.50	None
502	B M	16 mo.	10 kg.	55	1.83 g. ClO_2					
				15	Gluten from same flour	186	252	248	−0.35	None
538	F M	14 mo.	6 kg.	55	1.83 g. ClO_2					
				15	Gluten from same flour	186	214	194	+0.05	None
604	F M	4 mo.	5.15 kg.	70	0.715 g. ClO_2	355	116	103	+4.1	None
327	F F	9 yr.	6.75 kg.	70	0.715 g. ClO_2	236	206	197	+0.95	None
654	F F	8 mo.	5.35 kg.	70	50 g. ClO_2	155	210	176	+0.8	None
663	B F	20 mo.	7.9 kg.	70	50 g. ClO_2	156	181	157	+0.05	None
604	F M	3.4 mo.	5.95 kg.	70	3 g. NCl_3	21	129	116	+0.5	Present
617	F F	3.4 mo.	1.52 kg.	70	3 g. NCl_3	21	70	64	+0.23	Present
222	Mongrel F	Mature	6.3 kg.	70	20 g. NCl_3	13	138	112	+0.1	Present
349	F F	Mature	6.0 kg.	70	Defatted flour treated with 30 g. NCl_3	10	122	64	−0.8	Present

[1] B indicates Beagle; F, Fox Terrier; M and F, Sex male and female.

dogs made fairly good gains in bodyweight and mature dogs were maintained satisfactorily, regardless of treatment. Two young dogs, 654 and 663, were maintained satisfactorily for a period of 155 days on the ration containing flour treated with 50 g. chlorine dioxide per hundredweight. Fresh horsemeat was added to the daily food of dogs, 596, 604, and 605, in order to encourage good food consumption when these dogs appeared to be suffering from mild bacterial infection.

The total amount of fresh horsemeat fed to each dog was 1.8, 2.45, and 0.3 kg., in order mentioned above. At the end of the tests all dogs, except 594 and 605, were examined by practicing veterinarians and found to be in satisfactory condition from the standpoint of general appearance, quality of coat, and alertness. Dog 594 was found dead on the morning of December 26, 1947. There was no evidence of struggle preceeding death. The daily record revealed that for 21 days the dog had a good appetite and her temperature was normal. However, on the 22nd and 23rd day the dog did not consume any food, but there appeared to be no abnormality. Postmortem examination indicated a marked congestion in the lungs, and pneumonia as the probable cause of death. Dog 605 showed symptoms of a low-grade mixed bacterial infection and died of pneumonia after the continuous feeding of the test ration for a period of 174 days.

Symptoms of canine hysteria were not observed in any of the twelve dogs fed flour treated with chlorine dioxide, even when the treatment was increased from 0.61 to 50 g. chlorine dioxide per hundredweight. However, dogs that received rations containing flour treated with nitrogen trichloride developed symptoms of canine hysteria; the number of days required to induce symptoms and the severity of the symptoms depended upon the degree of treatment. The nitrogen trichloride treatment varied from 3 to 30 g. per hundredweight. Dogs 604 and 617 developed hysteria at the end of three weeks' feeding, but recovered. Dog 604 was used subsequently for the feeding of flour treated with chlorine dioxide. Dog 222 developed severe hysteria on the 12th day and was destroyed on the 15th day, due to the severity of the symptoms. Dog 349 developed severe symptoms of hysteria on the 4th day and died the following day. Observations of a similar nature were made in other studies, but the details of these findings are omitted in this report since reports on similar studies appear in the literature.

Monthly blood counts (hemoglobin, red cell and white cell counts and Schilling differential counts) of the dogs remained normal during the tests. There were a few high white cell counts and shifts in the differential counts, but these were associated with bacterial infection, pneumonia, and reaction following the administration of canine distemper vaccine.

Temperatures of the dogs fed rations containing flour treated with various amounts of chlorine dioxide remained within the normal range during the tests. Some elevated temperatures were observed, especially in young growing dogs during the administration of canine distemper vaccine. Elevated temperatures were recorded for dog 605, due to mixed bacterial infection and pneumonia.

At the end of the tests, autopsies were performed on dogs 502, 539, 654, 663, and 604. The animals were sacrificed by bleeding. All organs, brain, liver, spleen, kidneys, lungs, adrenals, thyroids, and pancreas, were removed at once and preserved in standard preservatives. These organs were found to be essentially normal. In some instances slightly enlarged or congested organs were observed, but no gross abnormalities were found in any of the organs examined.[4]

Electroencephalograms were taken at the Bureau of Biological Research, Rutgers University, on dogs, 654, 663, 604, and 327, that were fed with flour treated with chlorine dioxide. Records obtained for the three dogs, 654, 663, and 604, showed no abnormalities. Dog 327, however, showed an abnormal pattern, but it was impossible to determine whether it was due to the diet fed or other unknown factors. Encephalograms were not taken on any of the dogs at the beginning of the tests.

The results reported in this paper and unpublished data from this laboratory confirm the previously published findings that the feeding of flour treated with nitrogen trichloride induces canine hysteria, not only in growing dogs, but also in mature dogs. The severity of symptoms depended upon the degree of treatment, and the number of days on the diet. Symptoms appeared suddenly and animals either died or recovered after a change in the dietary regime, depending upon the severity of the symptoms.

The feeding of flour treated with chlorine dioxide, however, did not induce symptoms of canine hysteria in any of the 12 dogs. Young dogs made fairly good gains in bodyweight and appeared to be in excellent physical condition throughout the tests. Mature dogs were maintained satisfactorily without showing clinical abnormalities, even when flour was treated with 50 g. chlorine dioxide per hundredweight.

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FACTORS AFFECTING THE COLOR OF MACARONI. I. FRACTIONATION OF THE XANTHOPHYLL PIGMENTS OF DURUM WHEATS[1]

G. N. IRVINE and J. A. ANDERSON

ABSTRACT

The carotenoid pigments of semolina made from good and poor varieties of durum wheat, and of macaroni, have been extracted. Partition between 90% methanol and petroleum ether indicates that the pigments are almost entirely hydroxy-carotenoids. Chromatograms on zinc carbonate were similar for all samples and contained two main zones. The most dependable values for the absorption maxima (499 and 470 mμ) of the pigment in the upper zone of the chromatograms, and other supporting evidence, indicate that it is Taraxanthin. Values for the pigment of the lower zone (504 and 473 mμ) indicated that it was not a single compound. Rechromatographing of a larger quantity of lower-zone pigment obtained from a further experiment yielded two zones identified as Isolutein (503.5, 473 mμ) and Xanthophyll (508, 476 mμ). Thus the lower zone of the original chromatograms is considered to be Xanthophyll (also known as Lutein) together with some Isolutein formed during chromatographing. Visual estimation and optical density measurements indicate that there is somewhat more Xanthophyll than Taraxanthin.

Certain varieties of durum wheat, notably Pelissier and Golden Ball, produce macaroni of inferior color. The semolinas made from these varieties contain adequate amounts of yellow pigment but lose a high proportion of it during processing into macaroni. Other varieties, such as Carleton and Mindum, lose less pigment and yield macaroni of better color. As part of an investigation of this difference in behavior, a study was made of the types and amounts of xanthophyll pigments in two semolina samples representing good and poor varieties. A sample of macaroni was also examined to ascertain if any of the pigment fractions were preferentially oxidized during processing.

The true identity of the yellow carotenoid pigments of durum wheats was first established by Markley and Bailey (2). They

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showed that xanthophylls and their esters were the principal components and that carotene was present in much smaller amounts than was originally believed. Munsey (3) later showed that carotene constituted less than 1% of the carotenoid pigments of durum wheat, and he agreed that the principal pigments were xanthophylls. Crystalline Xanthophyll was isolated from Hungarian wheat flour by Zechmeister and Cholnoky (6) who also found little indication of any other important pigment fractions. A successful fractionation of the xanthophylls of durum wheats has not yet been reported in the literature, and it is with this problem that this paper is concerned.

Markley and Bailey (2) and Munsey (3) both attempted to fractionate the xanthophyll pigments extracted from durum wheat but were unsuccessful in obtaining a separation. Since their work was done, considerable improvements in extraction and fractionation techniques have been made by Karrer and his associates (1). Application of these newer methods in this laboratory has now yielded evidence that durum wheat contains both Xanthophyll and Taraxanthin. The procedure involves partition of the carotenoid pigments between petroleum ether and 90% methanol, chromatographing of the xanthophyll fraction in dry benzene on zinc carbonate, and subsequent identification by means of absorption spectra. It would have been preferable to confirm the evidence by isolating the individual pigments as has been done by Zechmeister and Cholnoky (6) with other products. But, as neither time nor equipment were available for the large scale extraction that would be required, it seemed preferable to forego such work in favor of other aspects of the main investigation.

Materials and Methods

When this study was undertaken, essentially as a side issue, only small samples of pure varieties were available for study. Absorption spectra of extracts of samples of Mindum and Stewart showed identical maxima, and as these varieties are of similar parentage it was considered legitimate to combine them to obtain a larger representative sample of good macaroni-making quality. Pelissier was available as a representative variety of poor quality. Semolina was made from both samples by the usual laboratory mill procedure. A reasonably large sample of macaroni made from pure Mindum was also available. It was ground to pass a 60 mesh sieve to yield a sample of similar granulation to the semolinas.

The method outlined by Karrer *et al.* (1) was followed in detail. The adsorption column used for the chromatographic analysis was 8 inches long and ¾ inch in diameter. The extent of development was the only variable among experiments with the three samples investi-

gated. This was varied in accordance with the amount of pigment obtained for chromatographing and also, to some extent, as additional experience was gained with the technique. The size of the samples extracted were: good variety, 500 g.; poor variety, 600 g.; and macaroni, 1,000 g. The samples were examined in this order, and the development of the chromatograms increased with each successive sample. The pigments in the bands were eluted with methanol and absorption spectra were determined in carbon disulfide with a Beckman Spectrophotometer.

In order to obtain enough material for rechromatographing of the principal pigment zone, a further study was made with 1,500 g. of semolina milled from a sample of 3 C.W. Amber Durum containing a mixture of varieties. This experiment is discussed separately.

Results and Discussion

The method involves extraction of the original material with methanol and with petroleum ether. After suitable treatment, the pigments in these two extracts are partitioned between 90% methanol and petroleum ether so as to separate the xanthophylls from the other carotenoids. With each of the three samples, all but a trace of the pigments were found in the methanol layers. That is, the great bulk of the pigments are xanthophylls. These results are in agreement with those of Markley and Bailey, and of Munsey.

The three chromatograms were almost identical in appearance. Each exhibited four bands. The uppermost band was only about one millimeter in depth, and was brownish in color. In all cases it was very tenaciously adsorbed and did not move down the column during development of the chromatogram. There was a clear region between this strongly adsorbed layer and the next colored band (zone 2). The second band was reasonably well defined, about five millimeters in depth, and yellow-orange in color. This band was followed by a region of a few centimeters depth which had a very pale yellowish color. This was not a region of strong adsorption, but appeared to contain mostly pigment washed down from zone 2. The final band (zone 4) was also reasonably well defined, slightly deeper than zone 2, and orange in color. Zones 2 and 4 contained the bulk of the pigments.

No quantitative determinations were made of the relative concentrations of the pigments of zones 2 and 4 of these chromatograms, but from a visual assessment of the relative depth and intensity of these bands, it appeared that the lower band (zone 4) contained slightly more pigment than the upper band (zone 2). This ratio appeared to be the same for all three chromatograms.

The amounts of pigments in the individual zones were too small

to permit further purification. This would involve rechromatographing, crystallization of the pigment, and final identification by melting point, optical rotation, and absorption maxima. In the present study only the absorption maxima could be determined. These are shown in Table I. The following discussion deals almost wholly with zones 2 and 4 which contained the bulk of the pigments. Three questions arise: Are the pigments obtained in each of the three experiments identical? If so, which experiment yields the most accurate data? And do these data agree with recorded maxima for known pigments?

TABLE I

ABSORPTION MAXIMA (mμ) FOR PIGMENTS ELUTED FROM EACH CHROMATOGRAM ZONE

Zone[1]	Semolina		Macaroni Mindum (Exp. 3)
	Mindum-Stewart (Exp. 1)	Pelissier (Exp. 2)	
1	498 469	415 383	381
2	501 471.5	498.5 469.5	499 470
3	500 470	500 470	500 470
4	504 473.5	503.5 473	503 472

[1] The zones are numbered from the top of the column.

Since the Beckman instrument yields data that are accurate within less than ± 0.5 mμ, the principal consideration is the purity of the pigment in each zone. This depends primarily on the efficiency of development of the three chromatograms, which was poorest for experiment 1 and best for experiment 3. Accordingly, it is believed that the pigment in zone 2 was obtained in purest form from experiment 3. The uppermost zone, which was invariably adsorbed tenaciously, was washed free of all pigments adsorbed lower, and zone 2 was also washed free of pigments adsorbed in zone 4. Almost as much development probably occurred in experiment 2 for which the zone 2 data are similar to those of experiment 3. But in experiment 1, it appears that zone 2 was not washed free of zone 4 pigments and therefore yields higher results. The opposite situation holds for the pigment of zone 4; it was least contaminated with pigments from zone 2 in the least developed chromatogram of experiment 1, and most contaminated in experiment 3. All data for zones 2 and 4 thus appear to be consistent with the hypothesis that all three samples contained

the same two principal pigments, one of which is adsorbed in zone 2 and the other in zone 4. The most reliable data for the absorption maxima are considered to be:

Pigment of zone 2 — 499 mμ and 470 mμ;
Pigment of zone 4 — 504 mμ and 473.5 mμ.

The pigment of zone 1 was very strongly adsorbed as was demonstrated by its failure to move down the tube at all during development. Accordingly, it is improbable that zone 2 was contaminated by any pigment from zone 1. But zones 2 and 4, and zone 3 which is thought to have been a mixture of pigments from zones 2 and 4, were less strongly adsorbed. Thus it seems probable that zone 4 was contaminated with some zone 2 pigment even in the least developed and certainly in the most developed chromatogram. Accordingly, though the maxima given above for zone 2 are probably reasonably accurate, those for zone 4 may be shifted towards the ultraviolet by contamination.

The known hydroxy-carotenoid pigments with absorption maxima similar to those of zone 2 pigments are:

Taraxanthin	— 499 mμ and 470 mμ;
Violaxanthin	— 500.5 mμ and 469 mμ;
Xanthophyll-monoepoxide	— 501 mμ and 472 mμ.

Maxima for the zone 2 pigment agree best with those for Taraxanthin. Additional evidence that the unknown is neither of the other two compounds was obtained by color tests. In ether solution, Violaxanthin gives a blue color with hydrochloric acid, whereas Taraxanthin does not. When treated with chloroform that has stood long enough to develop a slight amount of hydrochloric acid impurity, Xanthophyll-monoepoxide undergoes a molecular rearrangement with a shift in the absorption maxima to 479 mμ and 449 mμ (Karrer, 1). The zone 2 pigments (and those from all other zones) gave negative results for both tests. The pigment of zone 2 is therefore considered to be Taraxanthin, $C_{40}H_{56}O_4$.

Identification of zone 4 presents greater difficulty. Its maxima, 504 and 473.5 mμ, are close to those reported by earlier workers for Leaf Xanthophyll, 505 and 473 mμ. About 15 years ago Leaf Xanthophyll (or simply Xanthophyll) was thought to be one of two isomers; the other was called Lutein. The absorption maxima for these two pigments were given (5) as 505 and 473, and 508 and 475 mμ. Strain (4) has since suggested that Leaf Xanthophyll is a mixture, whereas Lutein is a single compound. The situation is further complicated because the pigment having maxima at 508 and 475 mμ is now called Xanthophyll by Karrer and his co-workers, whereas the name Lutein

is retained elsewhere. Since Karrer's methods were used in the present investigation, his nomenclature has been adopted.

The literature also shows that Xanthophyll is often difficult to separate, either by recrystallization or rechromatographing, from other similar pigments. Moreover, Strain (4) finds that on chromatographing *pure* Xanthophyll small amounts of a new pigment are formed. He calls this Isolutein and reports that it is adsorbed above the Xanthophyll and has maxima at 503 and 473 mμ.

These considerations suggested that rechromatographing of zone 4 might aid in its identification. A larger sample of semolina, milled from 3 C.W. Amber Durum wheat containing a mixture of varieties, was therefore treated by the method previously described. The initial chromatogram did not develop as effectively as had the other three, although a sufficient separation of zone 4 was effected. Rechromatographing of zone 4 on a smaller column yielded only one zone with maxima at 505 and 473 mμ. It was again removed and rechromatographed once more. This time, after prolonged development, two bands were obtained. The lower band exhibited maxima at 508 and 476 mμ which agree almost exactly with those for Xanthophyll, 508 and 475 mμ. The upper band had maxima at 503.5 and 473 mμ which again agree almost exactly with those for Isolutein, 503 and 473 mμ. Accordingly, zone 4 of all chromatograms is thought to consist mainly of Xanthophyll together with Isolutein formed on the column during the development of the chromatograms.

The relative amounts of pigment in the two main zones (2 and 4) of the initial chromatogram for the large sample of semolina were estimated from the optical densities of the pigment solutions. The ratio was one part of Taraxanthin (zone 2) to 1.35 parts of Xanthophyll (zone 4). This estimate must be considered approximate, but is also supported by visual inspection which suggested that there was slightly more Xanthophyll (zone 4) than Taraxanthin (zone 2) in each of the three original chromatograms.

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REACTION OF DOUGH AND GLUTEN WITH GLUCOSE [1]

I. HLYNKA and E. J. BASS

ABSTRACT

The application of the Chapman and McFarlane ferrometric reduction test to studies on dough and gluten shows that a reaction with glucose takes place. The following are typical experiments on materials stored for 5 months at laboratory temperature: Flour and flour mixed dry with 5% glucose had reducing values of 3.7×10^{-6} and 3.3×10^{-6} expressed as moles of ferrocyanide per gram sample, dry weight basis. Flour with 5% glucose, made into a dough, then air-dried and ground, showed a reducing value of 1.5×10^{-5} or a fourfold increase. Analogous experiments with gluten showed an eightfold increase in reducing value. It was also established that a storage period was necessary to bring about the glucose-protein interaction. Samples of flour with glucose, made into a dough, air-dried, and ground, showed no increase in reducing value over undoughed samples when assayed within several days. It was also shown that the reducing value of gluten was essentially unchanged by washing the gluten to remove the added and reacted glucose.

This evidence supports the hypothesis that reducing carbohydrates in dough and gluten may act as cross-linking agents between protein chains to form a three-dimensional elastomer network.

Observations on the behavior of dough and gluten towards such reagents as bisulfite and acetaldehyde led the writer (6) to suggest that a chemical interaction between wheat proteins and reducing carbohydrates was likely. To test this hypothesis, a method of measuring the carbohydrate-protein complex was necessary. One such method became available when Lea (9) established that the sugar-protein complex possessed reducing activity in the Chapman and McFarlane (2) ferrometric reduction test. This communication describes the application of the Chapman and McFarlane test to a study of dough and gluten.

Interest in the sugar-protein reaction, and a series of reactions initiated by it, has been wide and varied. In 1912 Maillard (10) noted that reducing sugars and amino acids reacted to yield a colored product which he termed melanoidin. Kostychew and Brilliant (8) observed a diminution in amino nitrogen on reacting protein hydrolyzates or amino acids with glucose. Further, the work of Frankel and Katchalsky (5) showed that this reaction was slow and could be

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determined titrimetrically. Bate-Smith and Hawthorne (1) associated the loss of glucose, and Olcott and Dutton (13) the appearance of brown discoloration, in dried egg products, with the condensation of glucose and protein. Also working with dried egg products, Pearce, Thistle, and Reid (14) developed a method of measuring the fluorescing substances which are formed. A similar interaction of cheese proteins with glucose was described by Hlynka and Hood (7) who produced a brown discoloration and observed changes in acidity in process cheese to which glucose was added. Finally, an important contribution to this subject has been made by Olcott and his co-workers (4, 11, 12). They established that aldehydes can act as cross-linking agents between functional groups in proteins and indicated that the cross-linking reaction is associated with the so-called browning reaction. It is now generally recognized that the complex series of reactions, initiated by the sugar-protein interaction, is of common occurrence especially where prolonged storage or heat processing is involved. Preliminary evidence is presented for the occurrence of this reaction between glucose, on the one hand, and flour or gluten, on the other.

Materials and Methods

Straight grade flour was experimentally milled from composite samples of Rescue and Thatcher wheat from the 1946–47 crop. Gluten was washed out by hand from Thatcher flour, air-dried, and ground to go through a No. 40 Wiley sieve. The following materials were prepared:

Untreated: Rescue flour, Thatcher flour, Thatcher gluten.

Dry mixtures of flour or gluten with glucose: Thatcher flour + 2% glucose, Thatcher flour + 5% glucose, Thatcher gluten + 2% glucose, Thatcher gluten + 5% glucose.

Mixtures with glucose of flour made into dough and gluten reconstituted with water, air-dried, and ground: Thatcher flour + 2% glucose, Thatcher flour + 5% glucose, Thatcher gluten + 2% glucose, Thatcher gluten + 5% glucose.

At the time of preparation of samples, the flour was about 6 months old. All the samples were then stored for about 5 months in the laboratory and reducing values were determined at the end of storage period.

Material was thus available for the determination of reducing values of flour and gluten, and of two types of flour and gluten mixtures with glucose. The first type contained dry glucose mixed with gluten or flour so that the effect of the presence of glucose on reducing value could be ascertained. In the second type of mixture, glucose with flour or gluten were wetted to make dough or reconstituted gluten,

and moisture was reduced to approximately that of the original products by air drying. These samples would thus allow reaction between glucose and flour or gluten under conditions similar to those in stored flour.

The method of determining reducing values is essentially that of Chapman and McFarlane, with several modifications. Since flour and gluten do not dissolve in the reagents used, but form a heterogeneous system, continuous mechanical stirring is necessary. A 1-minute period was adopted for the development of color with ferric chloride in the test sample. Lastly, since there is some turbidity in the final solution in which the color is measured, a blank identical with the test solution, but with an equivalent amount of water replacing the ferric chloride solution, was adopted to eliminate the influence of turbidity on colorimeter readings.

The procedure was as follows: To a suitable sample of flour or gluten in a 50-ml. centrifuge tube are added 5 ml. distilled water at 70°C. and the sample is stirred briskly into suspension. Then 5 ml. of potassium acid phthalate-sodium hydroxide buffer, pH 5, and 5 ml. of a 1% solution of potassium ferricyanide are added. Sometimes gluten will form lumps instead of a uniform suspension, but this difficulty is overcome if the water is added last. The centrifuge tube is then placed in a 70°C. water bath, and the same stirring rod that was used for agitation is now secured into the chuck of a stirring motor. The reaction mixture is heated at 70°C. for exactly 20 minutes with continuous power agitation, then transferred to an ice bath and allowed to cool to 25°C. Five ml. of 10% trichloracetic acid are added, the sample is centrifuged for 1 minute and filtered through a No. 1 Whatman filter paper. Five ml. of the solution are transferred to each of two colorimeter tubes. To one of these tubes are added 6 ml. distilled water and this tube is used as a blank with which the Evelyn colorimeter is set to read 100. To the second tube are added 5 ml. distilled water and 1 ml. freshly prepared 0.1% ferric chloride solution. One minute is allowed for the color to develop before reading is made. Colorimeter readings, made with a 660 mμ filter, are converted to reducing values in terms of moles of ferrocyanide by means of a calibration chart previously prepared. The final results are all reduced to a one gram, dry weight basis.

Results and Discussion

The experiments described may be divided into two parts, those with flour for which the data are summarized in Table I, and those with gluten for which the data are in Table II. The results are ex-

TABLE I

REDUCING VALUES OF FLOUR, VARIOUSLY TREATED

Expt. No.	Material	Treatment	Reducing value moles $K_4Fe(CN)_6$/g. $\times 10^{-6}$
1	Rescue flour	None	0.32
2	Thatcher flour	None	0.37
3	No. 2	At end of storage, wetted, air-dried, analyzed within 24 hours	0.36
4	No. 2 plus 2% glucose	Mixed dry, stored	0.37
5	No. 2 plus 2% glucose	Mixed, wetted, re-dried, stored	0.95
6	No. 2 plus 5% glucose	Mixed dry, stored	0.33
7	No. 2 plus 5% glucose	Mixed, wetted, re-dried, stored	1.50
8	No. 6	At end of storage, wetted, air-dried, analyzed within 24 hours	0.36

pressed as moles of ferrocyanide formed by the reducing action of the sample in the Chapman and McFarlane test.

An examination of the data in Table I shows that the reducing values for Rescue and Thatcher flour are nearly the same. Nor did 6 months storage or wetting and air drying (Experiment 3) have much effect. Experiments 4 and 6 establish that glucose does not act as a reducing substance in the Chapman and McFarlane test. However, when intimate contact was provided between flour and glucose by wetting, followed by air drying and storing the samples, as in Experiments 5 and 7, reducing values were greatly increased. The sample containing 2% glucose showed a 2.5-fold increase, and the 5% glucose sample a 4-fold increase. Finally, experiment 8 again shows that mere wetting of glucose and flour mixture and air drying it produces no marked increase in reducing value.

The results on reducing values in gluten experiments, summarized in Table II, are very similar to those of flour experiments. It will be

TABLE II

REDUCING VALUES OF GLUTEN, VARIOUSLY TREATED

Expt. No.	Material	Treatment	Reducing value moles $K_4Fe(CN)_6$/g. $\times 10^{-6}$
9	Thatcher gluten	Air-dried, stored	1.2
10	No. 9	At end of storage, wetted, air-dried, analyzed within 24 hours	1.2
11	No. 9 plus 2% glucose	Mixed dry, stored	1.1
12	No. 9 plus 2% glucose	Mixed, wetted, re-dried, and stored	6.5
13	No. 9 plus 5% glucose	Mixed dry, stored	1.1
14	No. 9 plus 5% glucose	Mixed, wetted, re-dried, and stored	9.8
15	No. 13	At end of storage, wetted, re-dried, analyzed within 24 hours	1.1

noted that gluten alone has a reducing value of about three times that of flour. Experiments 11 and 13 again show that glucose is not a reducing agent in the test, and Nos. 10 and 15 show that wetting and redrying of gluten also fail to enhance reducing values. Gluten, however, showed a higher reactivity with glucose than did flour. The 2% glucose sample (No. 12) showed a 4-fold, and the 5% glucose sample (No. 14) an 8-fold increase in reducing value compared with the original gluten. The reducing activity of these samples is apparently associated with the gluten, since washing gluten-glucose samples under the tap did not markedly decrease reducing values.

The inferences which may be drawn from the data are as follows. If Lea's conclusion, since confirmed by Crowe, Jenness, and Coulter (3), that the Chapman and McFarlane test measures sugar-protein complex formation is accepted, it follows that this type of reaction occurs between glucose and flour proteins. There is also the less likely alternative that it is the gluten lipids that react rather than the proteins. No specific data are available on this point.

Since small amounts of reducing carbohydrates are naturally present in flour it is logical that these should react with flour proteins in the same way as added glucose. Such interaction would be expected, especially in flour that has been stored for some time. Also judging from the high optimum water content which has been reported for this type of reaction, the reducing carbohydrate-protein interaction should be more pronounced in dough than in flour. However, doughs are not usually kept for long periods and the extent of the reaction might not be large. Nevertheless, the reducing property of the carbohydrate-protein complex suggests that it may be a hitherto unrecognized source of reducing activity in flour.

In summary, the results obtained in this investigation further support the hypothesis (6) that reducing groups in carbohydrates may act as cross-linking agents between protein chains in the elaboration of a three-dimensional network in dough and gluten. Such a hypothesis would thus relate the carbohydrate-protein interaction with the physical properties of dough and gluten in terms of their ultimate structure. Further studies on the reaction of glucose with gluten and dough are in progress to obtain further information on the questions raised in this investigation.

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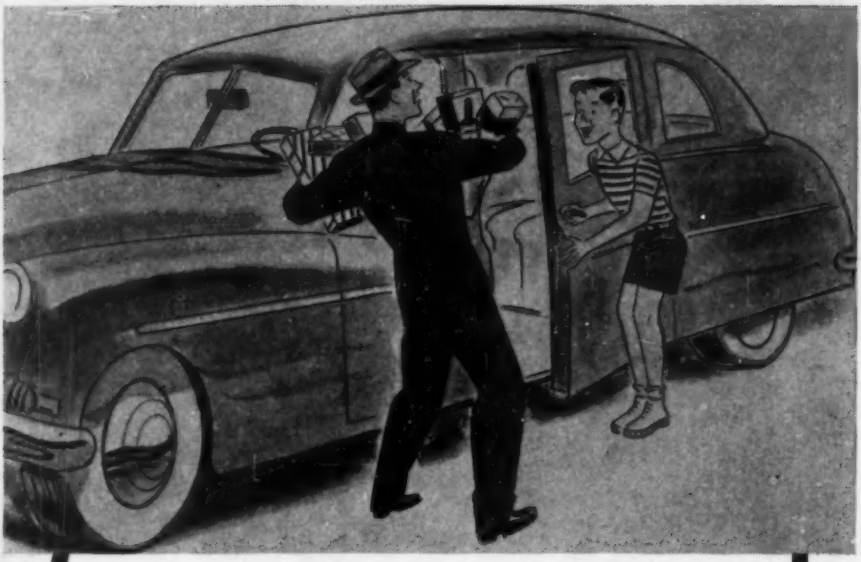

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